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EFFECTS OF ALFALFA SAPONINS ON DIGESTIVE FUNCTION IN SHEEP

BY

PETER T. KLITA

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE**

IN

ANIMAL NUTRITION

DEPARTMENT OF ANIMAL SCIENCE

EDMONTON, ALBERTA

FALL 1994

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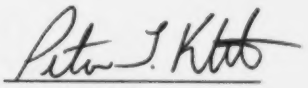

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **EFFECTS OF ALFALFA SAPONINS ON DIGESTIVE FUNCTION IN SHEEP** submitted by PETER T. KLITA in partial fulfilment of the requirements for the degree of Master of Science in Ruminant Nutrition.

G. W. Mathison

R. J. Christopherson

J. R. King

September 9, 1994

Dedication

This thesis is dedicated with love to my wife, Deborah L. Klita, in recognition of all the support she has offered me over the duration of my thesis program.

ABSTRACT

A Latin square design experiment was conducted with four ruminally and duodenally cannulated Suffolk wethers (60 ± 1 kg) to determine the effects of alfalfa root saponins on changes in forestomach motility, rate of digesta flow from the rumen, and microbial growth and efficiency. The sheep were fed a grass hay diet at a maintenance DM intake and alfalfa saponins were administered intraruminally in amounts equivalent to 0, 200, 400, and 800 mg/kg BW daily (0, 1, 2, or 4 % of DM intake daily), divided into two equal doses. Contraction frequencies during feeding tended to decrease in the rumen (P = .06) and reticulum (P = .11), and duration of reticular contractions tended to decrease (P = .11), on day 2 in response to added saponins. Longer administration of saponins (11 d) indicated an adaptation in factors controlling rumen contraction amplitudes since amplitude of contractions increased during resting (P = .06) and feeding (P = .05) by 79 and 102 %, respectively as the level of saponins increased from 0 to 4 %. No significant differences in frequency of contractions due to saponin treatments were detected on day 11 although numerical differences during feeding wee similar to d 2. Saponins caused increases in volatile fatty acid concentrations (P = .004) and lowered ruminal pH (P = .002) after 2 d of administration, however the compounds had no effect on day 11. On day 11, protozoal populations decreased (P = .005) and bacterial production linearly increased (P = .01) in response to higher levels of saponins. Increasing saponin levels resulted in increased duodenal flows of OM, NDF, and total N (P = .02, .09, and .01, respectively) and reduced apparent digestibilities of OM by 12 % in both the rumen (P = .02) and total digestive tract (P = .06).

Subsequently, a second experiment was undertaken to determine effects of higher saponin concentrations (as 4 and 8 % DM intake in a single dose) on forestomach motility in sheep. In two animals ruminal contractions were suppressed within 15 minutes and reticular contractions notably decreased at 30 min postdosing. At 3 h following saponin administration, the animal receiving an 8 % dose exhibited no forestomach motility for a 27 min period. Both animals failed to display feeding or rumination behavior for several hours following saponin administration. When one animal failed to recover from the saponin treatment, this experiment was terminated.

It was concluded that normal levels of saponins in alfalfa had a significant adverse initial impact on ruminoreticular motility, and that there was an adaptation of factors which control contraction amplitudes. The long term (11 d) effects of alfalfa saponins included a decrease in protozoal populations, increased bacterial N flow, and reduced ruminal and total tract digestibilities.

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List of Symbols, Nomenclature, Abbreviations

ADF	Acid detergent fibre
ADL	Acid detergent lignin
CNS	Central nervous system
DAPA	Diaminopimelic acid
DM	Dry matter
FOR	Fractional outflow rate
GC	Gas chromatography
GI	Gastrointestinal
GLM	General linear model
HPLC	High performance (pressure) liquid chromatography
HTMA	Hexadecyltrimethylammonium
I.D.	Internal diameter
MS	Mass spectroscopy
N	Nitrogen
NDF	Neutral detergent fibre
VFA	Volatile fatty acids

Chapter 1. Introduction

Plants provide the food we eat as well as an abundant source of compounds for use as food additives, pharmaceutical products, and a host of industrial applications (Oakenfull and Sidhu, 1989). Included in this group of compounds are saponins which, as the name implies, possess characteristics similar to those of soaps and detergents (Price et al., 1987). In addition, they also have an effect on biological activities which have implications for humans and animals.

Long before the identification of saponins, green twigs from saponin-rich plant species were used by Medieval Europeans and Aboriginal people to poison, in order to harvest, fish in water holes (Lower, 1985). Saponin-rich plants have also been the basis of many herbal remedies in European, Chinese, and Indian cultures. To-date, the identified activities of saponins include toxicity to fungi, fish, protozoa, insects, and some plants; inhibition of smooth muscle activity; inhibition of enzyme activity; altered cholesterol metabolism; and erythrocyte haemolysis (Cheeke, 1971; Bondi et al., 1973; Birk and Peri, 1980; Price et al., 1987; Oakenfull and Sidhu, 1989). Alfalfa saponins have been implicated as the primary factor involved in depressed growth in chicks (Heywang and Bird, 1954) and depressed egg production in laying hens (Heywang et al., 1959) when alfalfa meal was included as 20 % of the poultry diets. Swine exhibit reduced productivity when legumes such as alfalfa meal are major components of their diets, although they appear more tolerant to antinutritional factors than poultry (Cheeke, 1971).

Saponins have been demonstrated to act strongly as inhibitors of smooth muscle activity. This activity has been demonstrated on rabbit intestines, rat diaphragms and the muscles of the ruminant forestomach. Lindahl et al. (1957) showed that introduction of alfalfa saponins either intraruminally or intravenously could inhibit rumen motility in both sheep and cattle. In several experiments with oral or intravenous infusion of saponins, ruminant animals became severely ill, exhibited typical bloating characteristics, and either recovered slowly or were found dead within several hours of administration. Although saponins have been implicated in the etiology of bloat, some researchers (Majak et al. 1980, 1983, 1985) suggest that other plant components such as soluble proteins and chlorophyll exhibit a greater correlation with ruminant bloat than saponins. However, the activity of saponins on rumen motility has not been examined by most researchers and therefore this aspect of the effect of saponins on ruminant animals warrants further investigation.

Studies by Lu et al. (1982) and Lu and Jorgensen, (1987) suggested that saponins have a great influence on the microbiological and physiological systems of ruminants. *In vitro* studies (Lu et al., 1987) showed that saponin levels as low as 1 % of dietary dry matter (DM) reduced microbial nitrogen (N) outflow and total volatile fatty acid (VFA) concentration. In addition, these authors demonstrated that saponins, at physiological levels, increased total digestibility of organic matter and fibre in roughage and concentrate diets apparently by reducing apparent digestibility in the forestomach and by increasing digestibility in the small intestine. Also, saponins at 2% of the diet reduced dietary protein degradation in the forestomach, reduced microbial production, and increased

available protein in the lower digestive tract. There was a greater impact of saponins in diets based on roughage than in high concentrate diets.

It is apparent from the above discussion that further studies on alfalfa saponins are warranted. Previous investigations into alfalfa saponins have been performed with the acid hydrolysed compounds (Lu et al., 1987). This extraction procedure has been shown to yield sapogenins and saponin artifacts (Massiot et al., 1988), which are not an accurate representation of the structures present in forages. Also, the studies by Lindahl et al. (1957) were of relatively short duration, and no long term effects of alfalfa saponins on ruminoreticular motility have been determined. Thus, this study was performed to determine the short- and long-term effects of dietary alfalfa saponins on reticuloruminal motility, digesta flow, nutrient digestibilities, and conditions within the rumen of the ruminant animal.

Based on the information in previous literature, regarding the biological activities of saponins and effects of alfalfa saponins on a variety of animals, this study was designed to test the following hypotheses:

1. Alfalfa saponins are highly active inhibitors of smooth muscle activity and would suppress reticuloruminal activity at levels normally found in alfalfa. This influence on motility is expected to be observed as decreases in ruminal and reticular contraction frequency, amplitude, and duration.
2. Alfalfa saponins, through their influence on motility, would reduce particulate digesta flows in the ruminant animal.

3. Alfalfa saponins, through their actions on cell membrane cholesterol, will suppress rumen protozoal populations. This may have further effects on total microbial populations, feed digestibilities, and the rumen environment.

Chapter 2. Literature Review

2.1. Saponins

2.1.1. Structures. Saponins are glycosidic compounds composed of a steroid (C_{27}) (Mahato et al., 1982) or triterpenoid (C_{30}) (Kulshreshta et al., 1972) sapogenin nucleus (Fig. 2.1) with one or more carbohydrate branches, each branch containing some combination of monosaccharides. Monosaccharides include, but are not limited to, galactose, glucose, rhamnose, arabinose, and xylose. The triterpenoids are further divided into classes based on the compounds ursane, lupane, and oleanane (Bondi et al., 1973); the latter of which is most prevalent among alfalfa (*Medicago sativa* L.) saponins. The presence of oleane-12-ene sapogenins in the bark of *Quillaja saponaria* (Higuchi et al., 1988) and *Castanospermum australe* (Simes, 1950; Eade et al., 1963), berries of the Ethiopian plant *Phytolacca dodecandra* (Slacanin, 1988), leaves and twigs of the Mexican medicinal plant *Hypericum revolutum* (Décosterd et al., 1987) and *Oxytropis bicolor* (Sun ad Jia, 1991), and flours of pea (*Pisum sativum* L.) and soyabean (*Glycine max*) (Potter and Kummerow, 1954; Curl et al., 1985) as well as nearly all structures in alfalfa demonstrates that the occurence of these compounds is very diverse.

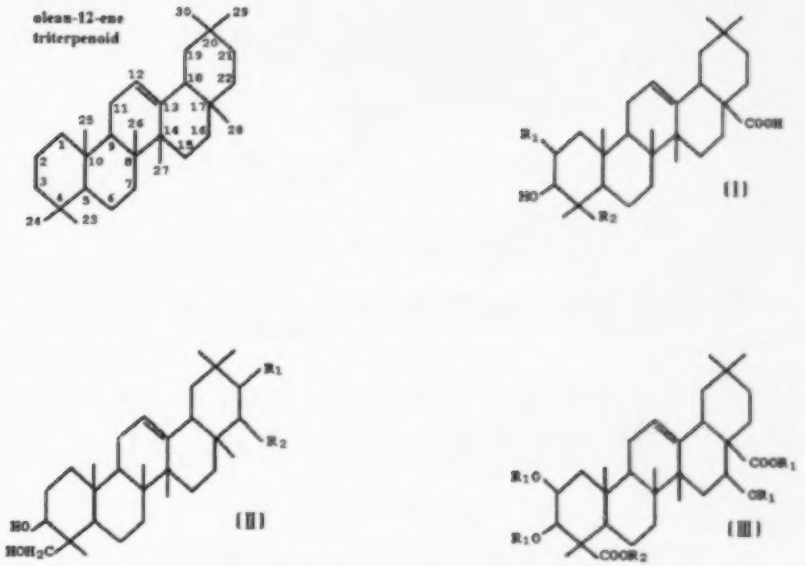
The triterpenoid sapogenins (Fig. 2.1) most commonly found in alfalfa include: soyasapogenols A, B, C, and E, hederagenin, bayogenin, lucernic acid, zahnic acid, and medicagenic acid; the latter considered to be most abundant genin. Among the first isolations of saponins common to alfalfa leaves and roots were the medicagenic acid 3-β-O-triglucoside (Gestetner, 1971) and compounds including hederagenin (Shany et al., 1972) which are present in North American alfalfa cultivars. Oleszek (1991) reported

that the most abundant alfalfa leaf and root saponin from Polish alfalfa cultivars is the medicagenic acid bidesmoside: 2-β-hydroxy-3-β-*O*-(glucuronopyranosyl) 28-*O*-[α-L-xylopyranosyl(1-4)-α-L-rhamonopyranosyl(1-2)-α-L-arabinopyranoside]Δ^{12}-olean-23-oic acid (3-GlcA,28-Xyl-Ara-Rha Ma). More recently however, Oleszek et al. (1992) reported that a zahnic acid tridesmoside contributes significantly to total saponins in alfalfa leaves and is the major saponin in some varieties: 3-*O*-[β-D-glucopyranosyl(1-2)-β-D-glucopyranosyl(1-2)-β-D-glucopyranosyl]-2β,3β,16α-trihydroxyolean-12-ene-23,28-dioic acid-23-*O*-α-L-arabinopyranosyl-28-*O*-[β-D-apiofuranosyl-(1-3)-β-D-xylopyranosyl(1-4)-α-L-rhamnopyranosyl(1-2)-α-L-arabinoside. Although not biologically active in it's original form, Oleszek suggested that partial degradation of this compound by microbial activity could leave a structure having similar activity to the medicagenic acid glycosides. Thus, the importance of this compound should not be overlooked. This compound was previously unknown and is the largest alfalfa saponin discovered so far with a molecular weight of 1678. Discrepancies in relative abundance of alfalfa saponins demonstrate the differences observed between cultivars and variation of saponin structures identified in various forages (Table 2.1).

Biosynthesis of saponins suggests that both triterpenes and steroids originate from the common precursor squalene; the origins of which are acetic acid converted first to mevalonic acid, then to farnesyl pyrophosphate.

2.1.2. Sources. Saponins have been identified in over 100 plant families and in 500 plant species. Although the majority of these plants are not used as food or feed

sources, commonly used saponin-rich plants include beans (*Phaseolus vulgaris*), spinach (*Spinacia oleracea*), oats (*Avena sativa*), potatoes (*Solanum tuberosum*), onions (*Allium cepa*), tea (*Camellia sinensis*), peanuts (*Arachis hypogaea*), sunflowers (*Helianthus annus*), guar (*Cyamopis tetragonoloba*), lupine (*Lupinus spp.*), and alfalfa (*Medicago sativa*) (Oakenfull and Sidhu, 1989) at concentrations ranging from trace quantities to nearly 30 percent (Fenwick and Oakenfull, 1981, 1983). Saponins have been extracted from virtually all plant parts including flower petals (Morris and Hussey, 1964), leaves, stems, and roots. Most saponins are concentrated in plant surface tissues (Simes, 1950; Quazi, 1975, 1976), and in alfalfa, are concentrated in shoot, root, and leaf tissues (Gestetner, 1971; Shany et al., 1972).



Abbr.	Sapogenin	Fig	R_1	R_2
Ma	Medicagenic acid	I	OH	COOH
H	Hederagenin	I	H	CH_2OH
B	Bayogenin	I	OH	CH_2OH
Sa	Soyasapogenol A	II	OH	OH
Sb	Soyasapogenol B	II	H	OH
Sc	Soyasapogenol C	II	>=<	
Se	Soyasapogenol E	II	H	>=O
Za	Zahnic acid	III	H	H

Figure 2.1 Sapogenins associated with alfalfa (*Medicago sativa* L.)

(adapted from figures in Massiot et al., 1988)

Table 2.1 Saponins identified in alfalfa (*Medicago sativa*) and similar structures found in other plants

Genin[a]	Carbohydrate moiety[b]	Sources[c]	References[d]
Ma	3-Glc, 28-AraRhaXyl	alfalfa leaf/root	Massiot et al., 1991
			Oleszek, 1991
			Oleszek et al., 1990, 1992
Ma	3-GlcA, 28-AraRhaXyl	alfalfa leaf/root	Oleszek, 1991
			Oleszek et al., 1990, 1992
Ma	3-GlcAraRha, 28-Ara-RhaXyl	alfalfa leaf	Massiot et al., 1991
Ma	3-GlcGlc, 28-AraRhaXyl	alfalfa root	Oleszek et al., 1990
		alfalfa leaf	Oleszek et al., 1992
			Massiot et al., 1991
Ma	28-AraRhaXyl	alfalfa leaf	Massiot et al., 1991
Ma	3-Glc, 28-Glc	alfalfa leaf	Oleszek et al., 1990
			Oleszek, 1991
Ma	3-GlcA, 28-ApiRha	alfalfa leaf	Oleszek et al., 1992
Ma	3-GlcGlc	alfalfa root	Massiot et al., 1988
Ma	3-Glc	alfalfa leaf	Oleszek et al., 1990
			Oleszek, 1991
		alfalfa root	Levy et al., 1986
Ma	3-GlcA	alfalfa root	Oleszek et al., 1990
H	3-AraGlcAra	alfalfa leaf	Oleszek, 1991
		alfalfa root	Levy et al., 1986
H	3-GlcGlcGlc	P.dodecandra	Slacanin et al., 1988
H	3-GlcAra	Honeysuckle	Curl et al., 1985
Sb	3-GlcAGalRha	alfalfa leaf	Oleszek, 1991
			Oleszek et al., 1990, 1992
		soya-bean	Ireland and Dziedzic, 1986
		pea	Domon et al., 1984
Za	3-GlcGlcGlc, 23-Ara, 28-AraRhaXylApi	alfalfa leaf	Oleszek et al., 1992

[a]Sapogenins described in Figure 2.1
[b]Carbohydrate chains identified by carbon position and monosaccharide units attached: GlcA=Glucouronic acid, Glc=Glucose, Rha=Rhamnose, Xyl=Xylose, Api=Apifuranose
[c]Plant(structure) compounds were isolated from.
[d]Refer to literature cited.

2.1.3. Extraction. Saponins are characteristically easy to extract, but rather difficult to purify. Due to the chemical nature of this family of compounds, extraction from plant material can be effected by polar solvents including water, methanol, ethanol, or some combination of these. Basic purification procedures have changed little over the years (Jacobson, 1918; Lindahl et al., 1954; Walter et al., 1954; Thompson et al., 1957; Shany et al., 1970a; Natori et al., 1981; Massiot et al., 1991). Further purification of saponins has been accomplished through separation in liquid mediums of various organic solvents (Morris et al., 1961), precipitation following the addition of cholesterol (Lindahl, 1957), or purification via column chromatography (Van Atta et al., 1961). These methods, although useful, are only capable of purifying saponins on a small scale. Saponins isolated by the above means, have provided some means of gravimetric quantification; albeit through very laborious procedures.

2.1.4. Properties. Purified saponins appear as either pale yellow or white crystals. Owing to the wide variety of potential carbohydrate groups, the sapogenins are more commonly characterized. Since identifying medicagenic acid (Djerassi et al., 1957), $C_{28}H_{42}(OH_2)_2(COOH)_2$, it is referred to as a dihydroxy dicarboxylic acid exhibiting a sharp melting point at 352-353°C and giving an $(\alpha)^{25}_{D}$ of +106° in absolute ethanol (Morris, 1961).

Saponins have been identified as compounds that produce a stable foam when agitated in aqueous solution. Due to the combination of fat-soluble sapogenin and water-

soluble carbohydrate components, these compounds are highly surface-active. Characteristically bitter, saponins have been recently been described as the most bitter compounds isolated which also leave a foul aftertaste (Oleszek et al., 1992). This property may explain inappetance in swine and poultry while rabbits show a feed preference towards bitter compounds. The saponin, glycyrrhinic acid, isolated from licorice roots is perceived to be 50 times sweeter than glucose, suggesting that bitterness is not a definitive characteristic of the presence of saponins. Due to their structure, saponins actively bind to other compounds; and will form insoluble complexes with cholesterol.

2.1.5. Biological activities. Saponins are a diverse group of plant compounds with a wide range of biological activities. Activities associated with saponins from major forage sources include erythrocyte haemolysis, lowering of blood and liver cholesterol levels, growth reduction, ruminant bloat, smooth muscle inhibition, enzyme inhibition, and altered nutrient absorption.

Triterpenoid saponins have been associated with enzymatic inhibition of cholinesterase in erythrocytes (Shaw and Jackson, 1957), and succinate oxidase in rat diaphragm (Cheeke and Oldfield, 1970). Inhibition of digestive enzyme activity by triterpenoid saponins *in vitro* (Ishaaya and Birk, 1965) has been reported for cholinesterase, chymotrypsin, and trypsin. Triterpenoid ginseng saponins inhibit cellular respiration, apparently by direct interaction with the membrane bound enzyme Na+,K+-ATPase from dog cardiac sarcolemma (Lee et al., 1986). Such activities could have

drastic effects in biological systems.

Reactions with cholesterol in the erythrocyte cell wall, resulting in increased permeability, may be responsible for haemolytic activity (Glauret et al., 1962); although not all haemolytic saponins form cholesteroids (Jones and Elliot, 1969). Affinity for cholesterol could also explain saponins toxic effects on fungi.

Saponin affinity towards cholesterol (Bangham and Horne, 1962) and bile acids has shown effects on cholesterol metabolism in monogastric species. Griminger and Fisher (1958) first suggested that lowered plasma cholesterol levels were related to saponin concentrations in chick diets. Saponin complexes caused greater bypasses of dietary cholesterol and prevented reabsorption of bile acids. Newman et al., (1958) also found that simultaneous addition of cholesterol and saponins to chick diets did not alter serum cholesterol levels.

Studies, showing that saponins are generally 50 times less toxic orally than when given intravenously (Lindahl et al., 1957), suggest that these compounds are absorbed very slowly, if at all from the digestive tract. Further studies on saponin digestion and absorption of saponins in chicks, rats, and mice failed to detect saponins in the blood (Gestetner et al., 1971).

Triterpenoidal saponins from alfalfa have been characterized as inhibitors of smooth muscle activity (Dougherty and Lindahl, 1957; Jackson and Shaw, 1959; Cheeke, 1989). This activity has been reported on rat diaphragm (Jackson et al., 1959; Shaw and Jackson, 1959; Jackson et al., 1962) and the rumen of sheep (Lindahl et al., 1957; Coulson and Davies, 1962). The inhibition of ruminal motility by alfalfa saponins has

provided sufficient evidence to support the theory that these compounds predispose ruminants to bloat by inhibiting eructation and normal forestomach motility (Lindahl et al., 1957; Jackson et al., 1959). Thus, saponins have been considered as antinutritional factors in legumes (Cheeke, 1985).

Studies with ruminants suggests that some rumen microflora are capable of degrading saponins (Gutierrez et al., 1958; Gutierrez and Davis, 1962), which limits any potential effects on blood cholesterol levels in these species.

Saponins in monogastric diets have caused depressed growth by some unidentified means. However, the influences on nutrient absorption may play a role as an antinutritional factor. Johnson et al. (1986) tested the effects of three triterpenoidal saponins and one steroidal amine glycoside on intestinal transport in vitro. In the presence of the triterpenoid *Gypsophylla* saponin, carrier-mediated galactose transport was inhibited; although the uptake of the passively transported L-isomer of glucose increased. Also, in contact only with the mucosal surface, *Gypsophylla* saponin caused a rapid decline in glucose-stimulated transmural potential difference, the response increasing as the saponin concentration increased from 0.3 to 8 mM. Similar responses to transmural potential difference were reported in the presence of *Saponaria* saponin and α-Tomatine, but soya saponins were much less effective. Johnson et al. (1986) indicated that some saponins readily increase the permeability of the small intestinal mucosal cells in rats, thereby inhibiting active nutrient transport, and facilitating the uptake of materials to which the gut would normally be impermeable.

Saponins as low as 0.1% of the diet have caused depressed growth in chicks

(Heywang and Bird, 1954), and levels of 0.26% depressed egg production in laying hens (Heywang, 1959). However, these effects can be overcome by addition of 1% cholesterol (Anderson, 1957) to the diets. Reduced growth has been observed in swine, although at higher saponin levels than observed in poultry. Levels of 2-3% alfalfa saponin were required to observe growth depression in rats (Coulson, 1957).

Bloat has long been considered a condition which occurs in ruminants partially due to the presence of saponins. Jacobson (1911, 1918) suggested that plant compounds may be involved in the development of ruminant bloat. Many saponin activities and characteristics lend themselves well to predisposing factors of bloat including: production of a stable foam, high surface-activity, alteration of microbial activity, and ability to inhibit smooth muscle activity. Greater stability of saponin-induced foams occurs at lower pH's (Mangan, 1959), which correspond to conditions in the rumen where the pH normally rages from 5.5-7. Lindahl et al., (1957) induced bloat in sheep and steers following oral or intravenous doses of saponins isolated from alfalfa. In experiments carried through 1953-55, Lindahl introduced saponins intraruminally as a 1 L solution or by intravenous drip of a 20-50 mL solution to mature sheep that were grazing on either clover, alfalfa, or grass hay pastures. In all situations, the onset of bloat was observed within 15 to 45 minutes following dosing, although the duration of symptoms varied between 1 to 4 hours. Intraruminal administration of 15 g saponins in sheep generally led to slight bloat in animals grazing legume pastures and no bloat in animals on grass hay. Administration of 25 g saponins led to slight or moderate bloat for 1 to 3 hours with eventual recovery of the animals. Moderate or severe bloat was observed with saponin

levels of 50 to 100 g in animals, regardless of their pretreatment diet. In severe cases, animals collapsed and died in 1 hour to 3 days; despite attempts to treat the animals. Following intravenous infusion of 5 g of saponins, sheep developed moderate bloat, collapsed, and died in 1 hour and 15 minutes. Intravenous infusion of 0.5 - 1 g, however; did not lead to the appearance of any bloat symptoms.

The study by Majak et al. (1980) in which high and low saponin alfalfa varieties were fed to cattle did not show significant differences in occurrences of bloat, and therefore did not provide evidence to support the saponin theory. Rather, through subsequent studies (Majak et al, 1983, 1985); it was suggested that there was a greater relationship between the chlorophyll and soluble protein levels than saponins of alfalfa with the incidence of alfalfa pasture bloat. However, a re-evaluation of alfalfa saponins on ruminant digestion (Lu et al., 1982; Lu and Jorgenson, 1987) suggested that observed microbiological and physiological changes could lead to bloat in sheep in response to dietary alfalfa saponins.

2.1.6. Uses and applications. Saponins have been beneficial in the food and drink industries, pharmaceutical applications, as well as general industrial applications (Oakenfull and Sidhu, 1989). The potential application of saponins for prevention of cardiovascular disease is appealing and supported by studies where primate serum cholesterol concentrations have been reduced by dietary saponins (Malinow et al., 1978, 1987). This reduction is achieved both by increases in fecal excretion of cholesterol and limited reabsorption of bile acids.

Saponins have been added to manufactured foods and drinks as foaming agents and emulsion stabilizers. Saponins from certain gourds have been patented as antioxidants for food use (Takashi et al., 1986). Saponin-rich fenugreek seeds are used in pickling to inhibit fungal growth. Saponin-rich plant extracts are used in a variety of food additives: crude extracts of licorice and sasparilla are accepted as food flavourings in Australia, Britain and the U.S., while all saponins are prohibited in Spain and Morocco (Oakenfull and Sidhu, 1989).

A number of saponins or saponin-rich mixtures have been used as anti-inflammatory, antidiuretic, antipyretic, and analgesic agents, central-nervous-system (CNS) depressants, and in the treatment of ulcers. Saponins appear to influence blood pressure with various plant sources shown to be either hypertensive or hypotensive. Ginseng, used as an essential Chinese medicine, is a rich source of saponins. Triterpenes have actual or potential direct pharmaceutical uses, whereas steroidal saponins are more useful as starting materials for chemical synthesis of steroid hormones and related compounds.

Saponins have been used for numerous applications as surfactants due to their chemical stability in the presence of salts, acid or alkaline solutions. Shampoos may contain saponins at concentrations of .001 to 10 %. Saponins have been used in diazo copying materials, photographic emulsions, and fire extinguishers. Uses of saponins in biological waste-treatment plants has improved bacterial production of biogases.

2.1.7. Saponin analyses

2.1.7.1. Biological assays. Many assays based on their biological activities have

been developed for saponins. Interaction with cholesterol, responsible for the haemolytic activity in erythrocytes (Dourmashkin et al., 1962; Glauret et al., 1962), has led to quantitative analyses such as the erythrocyte assay (Jones and Elliot, 1969) and the haemolytic micromethod (Oleszek, 1990). Forage saponins have shown greatest toxicity towards *Trichoderma viride* (Steiner, 1965; Scardavi and Elliot, 1967), which has allowed development of microbiological fungal assays (Zimmer et al., 1967; Livingston et al., 1977). Saponins have been suggested as natural pesticides (Applebaum et al., 1969), which led to the development of a biological assay based on the toxicity to the red flour beetle (*Tribolium castaneum*) larvae (Shany et al., 1970b). Similarly, toxicity to fish has been the basis of fish assays used early in the 20th century. Saponin toxicity has also been observed on various plants, from which biological assays were developed based on germination inhibition in lettuce (Pedersen et al., 1967), cotton (Pedersen, 1965; Nielsen, 1960), and wheat (Wyman-Simpson et al., 1991; Waller, 1993) seeds. Although these biological assays cannot provide absolute quantitative analysis, they have been beneficial in terms of plant selection procedures and saponin estimations.

2.1.7.2. Analysis by GC/HPLC. Technical developments in gas and liquid chromatography have provided simpler and more accurate means of quantification of various compounds. Gas chromatography (GC) has limited application because it can only be used in separation of the non-polar aglycone part of the saponin and the derivitization of compounds is subject to potential losses. GC procedures have been developed for quantitative analysis of medicagenic acid in alfalfa (Brawn et al., 1981; Rao

and Bories, 1987). Medicagenic acid , after hydrolysis of the parent glycosides present in leaf protein concentrate, was estimated as its dimethyl ester, di(trimethylsilyl) ether on either 3% OV-117 on Chromosorb W or 3% SP-2250 on Supelcoport operating at 320°C. These procedures can be considered quantitative for saponins if the sapogenin:sugar ratio is known for the saponin in question. When coupled to a mass spectrometer (MS), the GC-MS analysis can provide useful structural information. Analysis of complete saponin molecules has been limited to the steroidal compounds of ginseng roots and soyabeans.

High performance liquid chromatography (HPLC) provides a means of separating both nonpolar aglycones and intact saponins using either normal or reversed-phase chromatography on derivatized or underivatized mixtures. Detection of saponins or sapogenins normally relies on absorption of ultraviolet light by the carbon-carbon double bonds with wavelengths ranging from 202 nm (Jiann-Tsyh et al., 1981) for underivatized compounds to 260 nm (Oleszek, 1990) for derivatized compounds. The HPLC methods are as varied as saponins themselves, but some procedures have been developed specifically for the study of alfalfa saponins (Massiot et al., 1988; Oleszek et al., 1990), whereas others have been developed for similar olean-12-ene saponins (Domon et al., 1984; Decosterd et al., 1987; Slacanin et al., 1988; Okuyama et al., 1989), or other triterpenoids (Kimata et al., 1979; Lin et al., 1981; Shimizu et al., 1983; Ireland and Dziedzic, 1986; Ahmad et al., 1990). General similarities between the methods include UV detection of underivatized compounds on reversed phase columns with either methanol or acetonitrile solvent systems.

2.2. Bloat

2.2.1. History of bloat. Bloat (acute tympanites) is identified as an excessive accumulation of fermentative gases within the reticulorumen which the animal is unable to expel through the normal process of eructation (Winkler, 1982). Normally, gas bubbles produced in the rumen fluid coalesce, separate from the rumen contents to form pockets of free gas above the level of the contents; and are finally eliminated by eructation (Reid et al. 1975). Most cases of ruminant bloat include the frothy type, occurring on legume pastures (alfalfa, red clover, white clover) (Kingsbury, 1964; Jones and Lyttleton, 1969), legume hay (alfalfa hay), or grazing winter wheat pastures (Reid et al. 1975). The occurrence of free bloat is less common and generally distinguished by a number of pathological lesions or physical blockage of the oesophagus. The condition of bloat has long been observed in domestic animals with perhaps the earliest known record dating back to A.D. 60 (Reid, 1965), and remains a significant factor in current animal production. Incidence is relatively common in dairy and beef cattle, but less frequent in sheep.

The economic importance of this condition is known throughout the world with average death rate due to legume pasture bloat in the New Zealand Dairy industry at 0.3-1.2% of the population (Reid, 1976). The Southern Great Plains region of the United States reports bloat as the major cause of death among stocker cattle grazing winter wheat (Howarth and Horn, 1984). Death rates are considered to be 2.5% of total stockers and as high as 20% on some pastures. In Canada, the highest risk of legume pasture bloat and alfalfa hay bloat are along the park belt of the Northern Great Plains region with

reports of bloat on 40% of the livestock farms in this area (Howarth, 1975). In 1973, death losses in western Canadian feedlots were about 0.5%, with one-half to two-thirds of these attributed to bloat (Canadian Feed Manufacturers Association, 1973).

2.2.2. Animal factors. Bloat may result from the physical blockage of the cardial orifice by solid objects including apples, corncobs, and turnips (Winkler 1982), or the entrapment of the gases within a stable foam (Clarke and Reid, 1969; Ayre-Smith, 1971, Majak et al., 1985). Cattle have displayed hereditary bloat, although this condition is primarily seen in dwarf cattle; and is thought to be the result of anatomical defects which interfere with the normal eructation process (Winkler, 1982). Some evidence exists that bloat susceptibility in individual animals is heritable, and symptoms can be induced following the transfer of rumen contents between animals (Clarke and Reid, 1973). Total exchanges of rumen contents between high-susceptible and low-susceptible animals has produced temporary exchanges of susceptibilities over a 24 hour period. Bloat foam may be inhibited or dispersed by salivary mucin (Fina et al., 1961; Mishra et al., 1967). However, Mishra et al. (1968) stated that certain rumen bacteria could be involved in bloat through their actions on salivary mucin. Other qualities of saliva suggest its role as a precursor to bloat. In addition to the buffering capacity of bicarbonate in saliva, it is available to the microbes as a source of carbon dioxide. It has been suggested that salivary muco-proteins are a minor source of surface-active agents which may contribute to production of stable foam.

Bacterial by-products, including volatile fatty acids, can contribute to favourable

conditions for bloat by lowering the pH of rumen fluid (Mangan, 1959). Rumen pH below 6.0 increases foam viscosity with a maximum at pH 5.5 to 5.7 (McArthur and Miltimore 1969). Foam production and stability were significantly negatively correlated with rumen-fluid fractional outflow rates 2 hours after feeding loose or pelleted alfalfa hay (Okine et al., 1989a). Studies of high and low bloat susceptible animals have indicated that the major difference existing between these animals is the volume of fluid in the rumen (Cockrem et al. 1983). Rumen fluid exchange experiments tested the effects of rumen fluid volume, rumen fluid origin, and host animal susceptibility on bloat severity. Bloat occurrence was primarily associated with the volume of rumen fluid and independent of the susceptibility of the host animal or the origin of the rumen fluid.

Research indicates that processes which affect the rumen-fluid volume and dilution rate are important factors in susceptibility to bloat. These factors could include water exchange between plasma and rumen fluid across the rumen wall, salivary flow, sodium and potassium in the feed, and kidney function.

2.2.3. Environmental factors. In temperate regions, the classical conditions of bloat occur with greatest frequency when animals are turned out either on rapidly growing legume pastures in spring and early summer or following a heavy frost in autumn, with animals developing a stable frothy foam in the ruminoreticulum (Hall et al., 1984). Although generally characteristic in fields of fresh herbage, bloat has been induced experimentally on hay diets (Lindahl et al., 1957). Recently, Hall and Majak (1991) reported that cases of bloat occur with greatest frequency on days with greater daily

temperature variations and temperatures below 10°C. These weather conditions alter plant composition by lowering percent dry matter and acid detergent fibre; while increasing chlorophyll, total nitrogen, and soluble nitrogen. These conditions have been suggested to influence alfalfa pasture bloat (Majak et al. 1985).

2.2.4. Plant constituents. Legume bloat occurs with greatest frequency on pastures (Minson, 1990) of alfalfa (*Medicago sativa* L.), red clover (*Trifolium pratense* L.), and white clover (*Trifolium repens* L.). Thus, these species have been identified as bloat-causing legumes. Among the bloat safe legumes are birdsfoot trefoil (*Lotus corniculatus* L.), cicer milkvetch (*Astragalus cicer* L.), arrowleaf clover (*Trifolium vesiculosum* Savi.), sainfoin (*Onobrychis viciifolia* Scop.), and crownvetch (*Coronilla varia* L.).

Studies by Majak et al. (1980, 1983, 1985) suggest that legume bloat is correlated to the concentrations of chlorophyll and soluble protein in herbage. Studies of high and low saponin alfalfa strains developed by Pedersen (1971) suggested no significant differences in bloat incidences (Majak and Howarth, 1980). However, higher rumen chlorophyll concentrations did correspond to the occurence of bloat (Majak et al., 1983). Majak et al. (1983) suggested that chloroplasts and other feed particles provide sites encouraging microbes to produce excessive gases entrapped by bouyant, frothy digesta. Soluble protein concentrations (Jones et al., 1970) were considered adequate to stabilize foams in frothy digesta; although these concentrations did not vary with the occurrence of frothiness (Majak et al., 1985). Hall and Majak (1991) did find higher chlorophyll and nitrogen concentrations in alfalfa on days when bloat was reported. However, since

chlorophyll and soluble proteins have shown limited correlation to bloat, other agents must also contribute to the condition.

Bloat safe legumes contain tannins which might prevent foam formation in the rumen (Kendall, 1964). The presence of condensed tannins (proanthocyanidins) was confirmed by others (Jones and Lyttleton, 1973; Sarker et al. 1976), who proposed that the antibloat activity of condensed tannins may occur through precipitation of the soluble forage protein foaming agents in the rumen. The general principle evolved from these studies is that bloat-causing legumes are susceptible to rapid digestion by rumen micro-organisms, while bloat-safe legumes are digested more slowly (Howarth and Horn, 1984). The antibloat activity of tannins probably occurs by inhibition of microbial digestion rather than by precipitation of soluble protein in the rumen fluid.

Investigations were performed to determine why legume species differ in their susceptibility to microbial digestion. Initial digestion of fresh legume leaves was divided into four events: bacterial colonization of the leaf surface, bacterial penetration of the epidermal layer, maceration of leaf tissue, and bacterial penetration of the mesophyll cell walls (Cheng et al. 1980). Bacterial colonization on the leaf surface occurred more rapidly with alfalfa than sainfoin, and bacterial proliferation around stomata clearly indicated a response to substances leaching from the stomata openings (Fay et al. 1981). Bacterial penetration of mesophyll cell walls in legume leaves occur by a general disorganization of the cell wall (Cheng et al 1980). Species differences in resistance to mechanical damage were correlated with thickness of the epidermal and mesophyll cell walls (Lees, 1984). Thicker cell walls may decrease the initial rate of digestion by

reducing mechanical damage during chewing, by prolonging the time required for microbial penetration, or possibly; by reducing the leaching of soluble nutrients from unruptured mesophyll cells. In vitro studies on thin tissue digestion with cellulase (Sant and Wilson, 1981) showed that cell walls of bloat-causing legumes were digested more quickly than those of bloat-safe legumes. Strains of alfalfa selected for slower and faster digestion were fed to sheep with resulting decreases in rumen fluid chlorophyll, soluble protein, and volatile fatty acids from the slower-digesting strain (Kudo et al. 1985).

2.3. Forestomach Motility

2.3.1. Sensory receptors. Luminal stimuli are important in the regulation of gastrointestinal motility. Electromyographic studies on anesthetized and decerebrate animals have provided valuable information towards the further understanding of motility control in the ruminant gastrointestinal(GI) tract. Together with studies in surgically altered animals, much has been learned about how luminal stimuli of tension, tactile, chemical, viscosity, and thermal changes regulate forestomach motility and digesta flow characteristics (Tsuda et al., 1989).

2.3.1.1. Tension receptors. Receptors lying in muscle are termed tension receptors. Studies of tension receptors in sheep show that the highest density is located around the reticular groove and in the medial walls of the reticulum and cranial sac of the rumen (Leek, 1969). These receptors are innervated with myelinated fibres. Receptors were found at lower densities in other regions of the reticulum and cranial ruminal sac, the

ruminoreticular fold, and cranial pillar of the rumen. These areas correspond to the locations in which Ash and Kay (1959) were able to encourage reflex salivation, rumination, and increased motility following manual stimulation.

Tension receptors produce a persistent slowly-adapting discharge which responds to tension changes in smooth muscle contractions and perhaps also to changes of humoral substances in the blood. The activity of tension receptors is increased by heightened tensions developed actively or passively in the muscle. These receptors are readily excited by deformation of the wall by probing the luminal surface, and are uninhibited by the local anesthetization of the luminal surface. Ruminoreticular tension receptors provide a potent excitatory drive to the gastric centres of the medulla oblongata (Leek, 1967). However, sheep with completely emptied ruminoreticulum maintained normal activity despite the suggested lack of receptor activity.

2.3.1.2. Epithelial receptors. Sensory receptors located in the lining of the GI tract are referred to as epithelial receptors. These receptors, located about 150 μm below the luminal surface, lay near the basement membrane of the ruminoreticulum (Leek, 1972). Epithelial receptors are excited by light mechanical and/or chemical stimuli applied directly to the epithelium. Unlike the tension receptors: epithelial receptors have little or no resting discharge, are sensitive to both mechanical and chemical stimulation, and are characteristically rapidly adapting to stimuli. Epithelial receptors have very low thresholds for mechanical excitation responding to stimuli incapable of affecting the deeply situated tension receptors.

Epithelial receptors with rapidly adapting mechanoreceptor properties also have chemoreceptor properties. In contrast to their short latency, rapidly adapting response to mechanical stimuli, epithelial receptors slowly adapt to chemical stimuli after comparatively long latency of a few seconds for low molecular weight substances like HCl, to over a minute for high molecular weight substances. In the ruminoreticulum, overproduction of volatile fatty acids (VFA) causes ruminoreticular stasis. This is not a pH effect per se since VFA show greater response at any given pH than lactic acid or mineral acids. Among VFA, butyric acid is much more inhibitory than acetic or propionic acid.

2.3.2. Control of primary contractions.

2.3.2.1. CNS responses. The effects of luminal stimuli to the reticulorumen and reticulorumen activity appear to be mediated by a vago-vagal reflex. Primary and secondary contractions of the reticulorumen are extrinsically mediated contractions depending on a vagal motor supply (Gregory, 1984). Contractions continue when the vagal afferent supply is removed, but the efferent supply is retained.

Reticular distention and stretch of the reticulorumen fold provide powerful excitatory stimuli for reticular and primary rumen contractions in the anesthetized animal (Gregory, 1984). Responses are variable in the conscious animal, although both actions will stimulate rumination behaviour. Gaseous insufflation of the rumen will linearly increase the frequency of secondary rumen contractions and eructation in cattle and sheep. However, excessive pressures will suppress motility. Although the precise location

initiating secondary contractions is not known, it seems that the effect follows excitation of the slowly adapting mechanoreceptors in the cranial sac. Although ruminal distention has profound effects upon reticuloruminal activity; removing rumen contents does not inhibit contractions, suggesting that distention is not critically important. Ruminal distention also increases motility of the caecum and other areas of the GI tract. This effect is blocked by reversible vagal anaesthesia, indicating a vagally mediated component.

2.3.2.2. Reflex mechanisms. Chronically vagotomized sheep, that are provided with intragastric nutrition, are able to develop coordinated contractions which occur almost simultaneously over the whole reticulorumen (Gregory, 1984); indicating that local reflex mechanisms offer some regulation of motility. These contractions are blocked by atropine and by lowering rumen temperature to 30°C and are evidently initiated by the myenteric plexus (Gregory, 1984). Contraction frequency varied with the level of rumen distention (Gregory, 1982), indicating that within the intrinsic plexus there are tension-sensitive mechanisms. In addition, there are intrinsic myogenic reticuloruminal contractions, at a frequency of 10-12 per min; which are unaffected by ganglion or muscarinic blockers (Leek, 1985). These contractions may influence extrinsic contractions as well.

It is possible that the inhibition of reticuloruminal motility by VFA could at least partly involve local neural reflexes. Ruminal VFA inhibits neurogenically mediated intrinsic contractions of chronically vagotomized sheep (Gregory, 1982). This could be

a nonspecific effect, blocking neural transmission or activity of tension-sensitive myenteric ganglia, due to the anesthetic properties of butyrate and propionate. Alternatively, this could indicate the presence of VFA-sensitive myenteric neurons with sensory nerve endings in the epithelial layer.

2.3.2.3. Humoral responses. Constituents of blood plasma have shown effects on reticuloruminal activity. Systemic VFA concentrations will increase prior to abolition of ruminal motility (Gregory, 1987). Jugular infusion of VFA will inhibit reticuloruminal motility similar to ruminal infusions. This demonstrates the existence of a direct central effect of plasma VFA in addition to the neural inhibitory effect of ruminal VFA.

Plasma insulin and glucagon levels rise with ruminal infusions of butyrate and propionate. Plasma glucose levels increase before abolition of rumen motility (Bowen, 1962). Since these changes inhibit forestomach motility they could add to the inhibitory effects of the VFA (Tsuda et al., 1989).

Other chemical stimuli could inhibit motility by exciting vagal epithelial receptors (Leek and Harding, 1975) or after absorption. Inhibited motility following infusion of Na_2CO_3 responded to plasma acid-base changes rather than ruminal pH (Clark and Lombard, 1951). Ruminal infusion of NH_3 inhibits forestomach motility (Bueno et al., 1977). This effect is not dependent on ruminal pH or mimicked by systemic application; but may result from diffusion of NH_3 into the intraperitoneal cavity (Chalmers and White, 1969). Infusion of NH_3 not only mimics inhibition of forestomach motility, but also changes in caecal motility as observed with ruminal infusions (Gregory, 1987).

Chapter 3. Materials and Methods.

3.1. Saponins

3.1.1 Source. To prepare an adequate supply of saponins for this trial, approximately 500 kg of alfalfa roots were collected from a recently ploughed and disced field of Beaver alfalfa (*Medicago sativa*) after harrowing in late October. Before drying, the plant material was sorted by hand to remove all excess dirt, weeds, and vegetative material. The remaining root material was packed into stackable plastic containers and dried in forced air drying rooms at 41-44°C for approximately 1 wk until the roots had turned brittle. The tough root material was then reduced to approximately 2.5 cm pieces by cutting with a forage harvester (Sperry New Holland, New Holland, PA). The chopped material was then ground in a large Wiley mill (Arthur H. Thomas Co., Philadelphia, PA) through a 1.27 cm screen and then reground through a 0.64 cm screen, leaving approximately 210 kg of root material.

3.1.2 Extraction. The extraction process was modified from the procedure described by Lu and Jorgensen (1987). Extraction vessels were prepared by welding two 204 L steel drums together and attaching a removable cover and a drain on the bottom face. Two such vessels were used to contain all of the root material. Inside the vessels, the drains were covered with approximately 40 layers of cheesecloth to allow for suitable filtration of the liquid extract without impeding flow. Vessels were filled with alfalfa roots, and covered with a methanol:water (80:20 wt/wt) solution which had been heated to approximately 65°C in a steam-jacketed vessel. The filtrate, dark brown in colour and

containing about 18% DM, was collected and methanol was distilled from the material for recycling. Roots were extracted with four or five washes until the final extract had less than 0.5% DM and was light yellow in colour.

The extract was concentrated by evaporating the methanol in a steam-jacketed vessel while maintaining a temperature of 65°C. The remaining thick dark syrup (approximately 80 L) was poured into shallow stainless steel trays (46.2 x 60.2 x 4.5 cm) under ventilated hoods and evaporation was encouraged by passing air over the surface. When the solution in the trays contained less than 5% methanol, as determined by GC, the remaining material was frozen at -20°C. Complete drying was achieved in a freeze dryer (Model 50-SRC Virtis, Gardiner, N.Y.) with condensers at -62°C, shelf heat at 20 °C, and a vacuum of 0.4 mm Hg. Samples had a tendency to expand, creating a loaf form. At least 7 days under these conditions were required to ensure complete drying of the samples. Once dry, samples were crushed manually within plastic bags, yielding 30.2 kg of a golden brown powder. This hygroscopic powder was stored at -20°C in sealed plastic pails until needed.

3.2. Animal Trial

3.2.1. Experiment 1. Continuous administration of low saponin doses.

3.2.1.1. Experimental design. Four rumen-fistulated and duodenally-cannulated Suffolk wethers were used in a Latin square design experiment. Animals received grass hay at 2% of body weight daily, fed semicontinuously at 4 hour intervals from an overhead feedbelt controlled by a timer (Chrontrol CT model, Lindburg Enterprises, Inc.,

San Diego, CA). Treatments consisted of saponins which were administered intraruminally twice daily (on days 1-14; day 0 represents the day prior to saponin administration) in amounts equivalent to 0, 100, 200, and 400 mg/kg BW (total daily saponin administration was 0, 1, 2, and 4% of DM intake). Since the saponin extract contained 50.6 % sucrose, sucrose was administered concurrently with saponins to provide a level of sucrose equivalent to the level in the highest dose. Alfalfa saponins and sucrose were administered intraruminally through a funnel twice daily at 0800 and 1700 h, in 200 mL 35-40 °C water. Fresh water and trace mineralized salt (>99.00% NaCl, 0.015% I, 0.01% Co, Sifto Canada Inc, Mississauga, ON) were provided ad lib. Each period was 14 days with 7 day of adjustment between periods.

3.2.1.2. Dilution and passage rates. Digestive markers prepared according to the procedures of Uden et al. (1980) were used for determination of liquid dilution rates and particulate rates of passage, as well as for digestibility determinations. Grass hay was passed through a 1 mm screen and mordanted with chromium (Cr). For digesta kinetic studies, 5 g of cobalt ethylenediaminetetraacetic acid (Co-EDTA) and 10 g of chromium mordanted to fibre (Cr-fibre) were dosed intraruminally on day 6 with subsequent collection of rumen fluid and duodenal digesta over the following 72 hours. Rumen fluid, for Co analysis, was collected through a strainer from the rumen ventral sac at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 14, 18, 24, 30, 36, and 48 hours post-dosing. Duodenal digesta, for Cr analysis, was collected 0, 2, 4, 8, 12, 18, 24, 30, 36, 48, 56, and 72 hours post-dosing via a gate inserted into the duodenal T-cannula.

3.2.1.3. Duodenal and faecal flow. For total digesta flow measurements, animals were given a priming dose of 18 g Cr-fibre (ground to pass through a 1 mm screen) and 9 g Co-EDTA on day 9. This was followed by 6 g Cr-fibre and 3 g Co-EDTA daily divided into three doses on day 10 and four doses on days 11 to 14. Faecal and duodenal samples were collected four times daily and pooled on days 13 and 14. Duodenal flows of protein, fibre, and organic matter were determined from chromium and cobalt concentrations of duodenal digesta. Bacterial N in duodenal digesta (pooled on days 13 and 14) was estimated from the diaminopimelic acid (DAPA) content and its content in rumen bacteria. Rumen fluid was collected at 6 hour intervals, immediately frozen, and pooled over 24 h on day 12. For isolation of bacteria, rumen fluid was thawed, shaken to homogenize samples, and centrifuged at 500 *g* for 10 minutes to remove feed particles and protozoa according to the procedure of Lu et al., (1982). The supernatant was then centrifuged at 20000 *g* for 20 minutes to sediment the bacteria. The bacterial pellet was washed twice with physiological saline (0.9% NaCl) and rinsed with distilled water, then lyophilized and analyzed for DAPA content.

3.2.1.4. Motility recordings. Measurements of the effects of saponin administration on frequency, duration, and amplitude of contractions in the rumen ventral sac and reticulum were made during 8 h recordings, using procedures described by Okine et al. (1989b), from approximately 0900 h to 1700 h continuously on days 0, 2, and 11. Recordings on these days allowed evaluation of the control conditions, short term and long term effects, respectively. Motility recordings were taken from the reticulum and

rumen ventral sac of two animals simultaneously with four pressure transducers (Gould Stratham Instruments Inc., Hato Rey, Puerto Rico) coupled to a four channel physiological recorder (Beckman Electronics, Schiller Park, Ill.). Each transducer was connected via 4 m of tubing (1.4 mm I.D.) to a water-filled rubber balloon with attached weights (120 g) to minimize balloon movement in the reticulorumen. Positioning of the water filled-balloons was achieved by guiding the apparatus through the rumen cannula (2.5 cm I.D.) with a copper tube; accurate placement was determined by the recordings of characteristic biphasic peaks in the reticulum and guiding by feel around the muscular cranial pillar into the rumen ventral sac. The height of the pressure transducers were adjusted to a level to approximate the balloon's location while the animals changed positions.

Motility parameters, including the frequency, amplitude and duration of rumen and reticular contractions during eating, ruminating, and resting, were measured manually from chart recordings. Frequency of contractions was determined as the average number of observed contractions per minute over the 8 h recording interval from 0900 h to 1700 h. Amplitude and duration (time from onset of first biphasic contraction to the end of the second for reticular contractions) of contractions were averaged over 20 representative contractions. Contraction duration was measured to the nearest second, and amplitude to the nearest mm Hg. From the 8 h recording period, motility values were interpreted as total number of contractions, total duration in minutes, and total amplitude in cm Hg for a continuous 8 h period.

3.2.1.5. Respiratory calorimetry. The effect of saponins on methane and heat production was studied on day 12 by the procedure of Okine et al. (1989a), using indirect calorimetry and respiration hoods described by Young et al. (1975). The DataGrabber data collection system, developed by Godby and Gregory (private communication), was utilized for the collection of data pertaining to respiratory rates, oxygen consumption, and carbon dioxide and methane production. Energy losses as methane were estimated based on respired methane. Animal heat production was calculated based on the McLean equation (McLean, 1972), which is based on the oxygen consumption values ($H = 4.89V_oX$; where H is heat production (kcal) and V_oX is O_2 consumption (L)).

3.2.1.6. Rumen collections and blood samples. Rumen fluid was collected at approximately 1400 h on days 0, 2, and 14 for subsequent analysis of volatile fatty acid (VFA) concentrations and protozoal counts. Rumen fluid samples were collected from the rumen ventral sac, strained, and 5.0 mL added to 1.0 mL of 25 % phosphoric acid in a sealed culture tube. These samples were immediately frozen, stored at -20°C, and 1.0 mL of internal VFA standard added prior to analysis.

Jugular blood was collected in heparinized vacutainers at the same time as rumen samples were taken, analyzed immediately for haematocrit, then centrifuged, and blood plasma was frozen.

3.2.2. Experiment 2. Single doses of larger amounts of saponins. Following the first experiment, a study was undertaken to determine the effects of higher saponin doses

on reticuloruminal motility. Animals maintained on the grass hay diet were dosed with saponins at either 800 or 1600 mg/kg BW (4 or 8 % of DM intake). Recordings were taken from 0900 h to 1500 h to allow for observation of initial resting conditions, feeding at 1000 h, saponin dosing at 1100 h, and animal responses immediately following administration. This study was not replicated due to the ill effects of one animal which was later euthanized.

3.3. Analytical Procedures

3.3.1. Saponin analysis. The saponin extract was analyzed by the haemolytic micromethod procedure of Jurzysta (1979) for the determination of saponin concentration. Briefly, 10 µl of an extract solution was spotted on a plate covered with a thin layer of blood and gelatin suspension. As a result of saponin diffusion, and the reaction with biological membranes, a haemolytic ring appeared. The diameter of the zone of haemolysis was compared to those of standard saponins from *Quillaia saponaria* (Number S-2149, Sigma Chemical Co., St. Louis, MO) for an estimation of saponin concentration.

3.3.2. Sugar analysis. A Varian 3700 series gas chromatograph (Varian,Sunnyvale, CA., U.S.A) equipped with a flame ionization detector and an SE-30 column (30 m x .25 mm I.D. x .25µm film thickness, Supelco, Belefonte, PA, U.S.A.) was used to analyze the alfalfa extract for simple pentose and hexose monosaccharides and disaccharides. The temperatures of the injector port and detector were kept at 270°C with the injector flow split at 15:1. The column was isothermal at 270°C. Helium was

used as the carrier gas, with head pressure of approximately 20 psi, at 30 mL/min. The flame detector was supplied with air at 300 mL/min and hydrogen at 30 mL/min. The peak areas were recorded and analyzed with the Shimadzu EZChrom Data System (Shimadzu Scientific Instruments, Columbia, MD, USA). Samples were treated with 50 µL TMSI(N-trimethylsilylimidazole) and 50 µL pyridine and heated in a hot water bath for 10 min prior to injection of 1 µL aliquots. C18:0 and C22:0 fatty acids were used as internal standards to quantify a variety of monosaccharides (xylose, glucose, mannose, galactose, rhamnose, arabinose) and disaccharides (sucrose, maltose).

3.3.3. Chromium and cobalt. Rumen fluid samples for cobalt analysis (Reese et al., 1994) were prepared by centrifugation at 5000 *g* for 15 minutes to sediment all particulate matter, and the supernatant was diluted with distilled de-ionized water to allow for direct measurement on the atomic absorption spectrometer (Model 4000, Perkin-Elmer, Norwalk, CT). Standards for these samples were prepared by diluting cobalt standards in distilled de-ionized water to provide final solutions of 0, 0.5, 1, 2, 4, and 8 µg/mL. Reticulorumen liquid dilution rates were determined by linear regression of the natural log values of cobalt concentrations after peak concentration over time. Liquid volumes were predicted by extrapolation of the regression equation to estimate cobalt concentration at time zero.

Duodenal and faecal samples for analysis of chromium were prepared with a modification of the procedure used by Reese et al. (1994). Digesta samples were dried for 3 d at 60°C prior to grinding through a 1 mm screen in a Wiley grinding mill (Arthur

H. Thomas Co., Philadelphia, PA). Samples were digested with 4 N HNO_3, then neutralized and complexed with EDTA in an ammonium hydroxide solution. Filtered samples were diluted or analyzed directly for chromium and cobalt at 357.9 and 240.7 nm respectively on an atomic absorption spectrometer.

3.3.4. Diaminopimelic acid. Samples were analyzed by HPLC for determination of DAPA which was used as a natural marker of bacterial protein. Analysis of DAPA was modified from the procedure of Dugan et al. (1992). Samples (50 mg bacteria or 150 mg feces or digesta) were hydrolyzed in 3 mL 6 M hydrochloric acid in a screw cap culture tube (16 h, 110°C) which had been purged with N_2. In addition to a preinjection Supelco 50 mm x 4.6 mm I.D. guard column (Supelco, Bellefonte, PA, USA), a precolumn silica gel (Whatman Biosystems, Maidstone, UK) was employed to protect C18 packing in post-injection columns. A binary gradient changing from a polar to a non-polar solvent was used for sample elution. The polar solvent consisted of a water-methanol mixture (60:40, vol/vol) containing 0.1 M sodium acetate and 7.5 mM HTMA (hexadecyltrimethylammonium). The non-polar solvent consisted of a methanol-water mixture (95:5, vol/vol) containing 7.5 mM HTMA. Solvents were adjusted to pH 6.4 with glacial acetic acid. Under these conditions DL-α-aminocaprylic acid, DD,LL-DAPA, and DL-DAPA were found to elute at 28.4, 33.4, and 35.3 minutes respectively. Peak heights and retention times were measured using a Shimadzu EZchrom chromatography data system (Shimadzu Scientific Instruments, Columbia, MD, USA).

3.3.5. Nitrogen analysis. Samples were analyzed for nitrogen using the LECO Model FP-428 nitrogen analyzer (St. Joseph, Michigan). Approximately 50 mg of bacteria or 80 mg of feed/digesta were prepared for analysis by weighing the samples directly into small (100 mg) tin foil cups, sealing the capsules, and compressing the samples into a tablet form. Samples were combusted completely and nitrogen content determined from the nitrogen concentration in the combustion gases.

3.3.6. Fibre analysis. Neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL) were determined using the methods described by Van Soest and Robertson (1980). NDF analysis was performed after preparing samples with the heat stable amylase (Number A3306; Sigma Chemical Co., St. Louis, MO) as suggested by Van Soest et al. (1991). Cellulose and hemicellulose concentrations were estimated from the subtraction of ADL from ADF and ADF from NDF, respectively.

Ruminal and total tract digestibilities were determined based on marker estimated duodenal and total tract digesta flows and fibre composition of these samples on a DM basis.

3.3.7. Energies. Gross energy values of the hay diets and extract were determined with a Parr adiabatic bomb calorimeter (Parr Instrument Company Inc., Moline, IL).

3.3.8. Protozoal counts. Protozoal populations in rumen fluid were determined directly from rumen fluid samples already diluted and prepared for VFA analysis.

Protozoa were counted with the aid of a Neubauer counting chamber (Clay-Adam, Parsippany, N.J.). Rumen fluid samples were shaken to ensure homogeneity. The counting chamber was cleaned with distilled water, dried, and prepared with a cover glass placed over the counting area with the edges resting on the cover-glass supports. Samples were transferred via a pipet to the edge of the cover slip allowing the 0.1 mm deep chamber to fill by capillary action, ensuring that there was no bubble formation under the cover-glass. The primary square of the counting chamber was visualized under the microscope at 40 x magnification. Owing to the relatively large size of the protozoa, a 1 mm x 1 mm area was designated as the counting area. Total protozoa numbers were recorded in duplicate for each sample and concentration expressed as protozoa per mL.

3.4. Statistical analysis.

The data was analyzed as a 4 x 4 Latin square design. Least squares means for main effects were computed with the general linear models (GLM) procedure (SAS, 1985). Linear and quadratic effects on treatment levels were computed using orthogonal contrasts in SAS. Relationships between attributes of rumen and reticular contractions with digestibilities and digesta flows were computed with the correlation (CORR) procedure (SAS, 1985), expressed as Pearson correlations.

Chapter 4. Results.

4.1. Feed composition.

There were no observable differences in hay composition during the four experimental periods (Table 4.1).

The saponin extract contained 27.8 % saponin, 50.6 % sucrose, 3.9 % ash, 1.9 % nitrogen, and .9 % crude protein (Table 4.2). The extract contained measurable, but insignificant, levels of crude fats, glucose, xylose, and arabinose; and undetectable levels of fibre and nucleic acids. Therefore the composition of 16 % of the extract remained unknown.

4.2. Experiment 1.

4.2.1. Animal health. Animals did not appear to be adversely affected by saponin treatments as indicated by their alertness and healthy appetites for the duration of the experiment. However, all animals showed increased seepage of ruminal contents, around the ruminal cannulae, on the highest levels of saponin administration. Animals readily consumed hay at maintenance level of feeding (2 % of BW) and feed weighbacks were infrequent.

4.2.2. Time spent eating, resting, and ruminating. Duration of resting, ruminating, and feeding activities during the 8 h recording intervals (0900 h-1700 h) on days 0, 2, and 11 are shown in Table 4.3. Saponins caused no differences in the time spent on any of these activities on days 0 or 11. However, time spent resting on day 2 increased (P =

.09) with increased saponin administration.

4.2.3. Frequency, amplitude and duration of reticulorumen contractions. Frequencies of rumen and reticular contractions during feeding, ruminating, and resting are shown in Table 4.4. Overall, the effects of saponins were variable with no significant differences other than reticular activity during feeding on day 0 (P=.03). However, strong tendencies towards reduced frequency on day 2 during feeding were present in the rumen (P = .06) and reticulum (P = .11). When data was expressed as a percentage change from predosing values (Appendix Table 7.7), reticular frequency was increased by 11 % (P = .02) on day 2 during the resting phase when 4 % saponins were administered and tended to be increased by 12 % (P = .10) during the rumination phase.

Amplitudes of contractions in the rumen and reticulum during feeding, ruminating, and resting are shown in Table 4.5. Amplitudes were not affected by saponins on days 0 and 2 for either the rumen or reticulum. On day 11 however; rumen contraction amplitudes increased linearly with saponins during resting (P = .06) and feeding (P = .05), by 79 and 102 % respectively, but not during rumination (P = .41). Reticular contraction amplitudes were highly variable. Differences between day 0 and days 2 and 11 contraction amplitudes were also not significant for the rumen or reticulum (Appendix Table 7.8).

Mean duration of rumen and reticular contractions as affected by saponins during resting, ruminating, and feeding are given in Table 4.6. Rumen contraction duration was

not related to administered saponins on days 0, 2, or 11 for the rumen, or on days 0 and 2 for the reticulum. However, reticular contraction duration showed a linear decrease (P = .06) of 5 % during feeding with increasing saponin doses. Also, a quadratic effect of saponins on duration of rumen contractions was observed on day 0 during rumination. Although contraction durations were variable in response to saponins, rumen contraction durations were significantly longer than the biphasic reticular contractions.

Further analysis of ruminal and reticular motility data provides information on the period and animal effects, 8 h motility values, and changes from day 0 for frequency, amplitude, and duration of rumen and reticular contractions (Appendix Tables 7.1-7.9).

4.2.4. Rumen liquid dilution and particulate passage rates. There was a tendency (P = .14) towards a linear decrease in duodenal particulate passage rates with increasing saponin levels on days 6-9 while duodenal particulate pool size tended (P = .12) to increase by 61 % (Table 4.7). The tendency (P = .14) for reduced particulate passage rate corresponds to a 17 % increase (P = .14) in mean retention time (MRT) for particulates between no dose and the high dose. Marker estimated particle outflow increased (P = .05) by 25 % with increasing levels of saponins. Results for ruminal liquid kinetics were variable, with no differences in liquid pool sizes (P = .49), passage rate (P = .79), or MRT (P = .96).

4.2.5. Duodenal flows and digestibilities. Duodenal flows, as determined from the Cr-mordanted fibre marker on pooled samples from days 13 and 14, are shown in Table

4.8. Although feed DM intake did not differ (P = .17) between saponin treatments, there were linear increases of duodenal flows of OM (P = .02), NDF (P = .09), ADF (P = .12), and N (P = .01) with increasing saponins. Bacterial N flow also tended (P = .13) to increase. Linear decreases in forestomach digestibilities of OM (P = .02), NDF (P = .05), ADF (P = .07), and N (P = .004) were observed with increased saponins, with similar tendencies in total observed digestibilities (P = .13, .15, .22, and .06, respectively). Other N (feed and protozoal) flow was not different (P = .97) between saponin treatmnents.

Marker estimated values of total tract digestibility consistently overestimated total collection values by 6-7 %.

4.2.6. Ruminal pH and concentrations of volatile fatty acids. Rumen pH values (Table 4.9) on days 0 or 14 did not differ, but showed a linear decrease on day 2 (P = .002) in response to increased saponin administration.

VFA concentrations in rumen fluid on days 0, 2, and 14 are given in Table 4.9. Initial concentrations did not differ for total, acetic, propionic, isobutyric, butyric, and valeric acids (P = .68, .31, .52, .17, .82, and .78 respectively), although caproic acid was lower (P = .04) and isovaleric acid tended (P = .07) to differ prior to administration of saponins. On day 2 of saponin administration, there were linear increases in total, acetic, propionic, and valeric acid concentrations (P = .003, .003, .01, and .02, respectively) and a tendency (P = .07) towards increased butyric acid with increasing saponins; while the branched chain fatty acids isobutyric and isovaleric showed linear decreases (P = .02 and .05 respectively). Total, acetic, propionic, and butyric acid concentrations did not differ

on day 14, but isobutyric and isovaleric acids continued to show linear decreases in concentration in response to saponins (P = .04, .01, respectively). Similarly, valeric acid continued to show a trend towards a linear increase (P = .09) in concentration with saponin dose on day 14.

4.2.7. Ruminal protozoal counts. Protozoal populations in rumen fluid did not differ (P = .48) prior to dosing saponins (Table 4.9). However linear decreases in population, with increased saponin dose, were highly significant on days 2 (P = .005) and 14 (P < .001). The lowest recorded protozoal population (1.2 x 10^4 per mL) was on day 2 with the highest dose. This concentration was less than predose concentrations by a factor of 10.

4.2.8. Haematocrit. Haematocrit values (Table 4.10) did not differ in response to saponin treatments on days 0, 2, and 14 (P = .68, .21, .67, respectively).

4.2.9. Calorimetry. There were no significant differences in oxygen consumption (P = .51), carbon dioxide production (P = .48), or methane production (P = .91) during measurements taken on day 12. Similarly, calculated animal heat production (P = .77) and energy lost as methane (P = .91) did not differ due to saponin treatments.

4.2.10. Relationships between reticulorumen contractions and total digestibilities. Correlations between ruminal and reticular contraction frequencies during corresponding

activities were highly correlated ($P < .001$) (Table 4.12).

Reticular contraction frequencies showed high positive correlation with total digestibilities of OM, NDF, ADF, and N during resting ($P = .02$), feeding ($P = .01, .01, .01, .02$, respectively), and total daily activity ($P = .02, .02, .02, .03$, respectively). However, duration of reticular contractions showed a high negative correlation with total digestibilities of OM, NDF, ADF, and N during resting ($P = .03, .03, .03, .02$) and total daily activity ($P = .02, .03, .03, .02$).

Ruminal contraction frequency was positively correlated ($P = .05$) with total ADF digestibilty during overall activity, but did not show significant correlation during individual activities. Ruminal contraction amplitude during rumination showed high positive correlation with total digestibilities of OM, NDF, ADF, and N ($P = .006, .007, .009, .003$, respectively). Daily ruminal contraction amplitudes showed a positive correlation with N digestibility ($P = .04$).

4.2.11. Relationships between reticulorumen contractions and duodenal flows. Reticular contraction frequencies during resting showed high positive correlation with duodenal flows (Table 4.13) of total DM, OM, total N, and bacterial N (Fig. 4.1) ($P = .02, .02, .01, .0002$, respectively). These correlations were also significant during rumination ($P = .003, .01, .001$, and $.002$), feeding ($P = .003, .004, .002$, and $.002$), and total activity ($P = .007, .008, .002, .001$). Contraction frquency during feeding showed positive correlation with duodenal flows of NDF ($P = .03$) and ADF ($P = .05$). Correlations of reticular frequency with DM, total N, and bacterial N flow are shown in

Figs. 4.2-4.4. Reticular contraction frequency was highly correlated with bacterial efficiencies (bacterial production per kg of apparently digested feed) during resting (P = .05), ruminating (P = .01), feeding (P = .05), and total activity (P = .001). Total number of reticular contractions observed during the recording interval on day 11 showed significant correlation to duodenal DM (P = .007) (Fig. 4.5) and N (P = .002) (Fig. 4.6) flow.

Reticular contraction amplitudes during day 11 were positively correlated with ADF flow (P = .03).

Duration of reticular contractions (Table 4.13) showed the highest correlation with digesta flows during resting. Contraction duration at rest was highly negatively correlated with duodenal flows of OM (Fig. 4.7), NDF, and ADF (P = .05, .01, .01); and indicated similar trends for DM, total N, and bacterial N (P = .10, .12, .08). Reticular contraction duration during rumination and feeding did not correlate significantly with digesta flow parameters or with bacterial efficiencies. However, total contraction duration during the recording interval was significantly correlated with duodenal flows of NDF (P = .04) and ADF (P = .03).

Ruminal contraction (Table 4.14) frequencies showed high positive correlation with duodenal flows of DM, OM, total N, and bacterial N during resting (P = .03, .02, .01, .002), feeding (P = .004, .01, .003, .01), and total activity (P = .06, .05, .02, .01). Ruminal contraction frequency also showed high positive correlation with duodenal flow of NDF during resting (P = .05) and feeding (P = .04). Ruminal frequency showed positive correlation with apparent bacterial efficiency during resting (P = .006), feeding

(P = .03), and daily activity (P = .03).

Ruminal contraction duration during feeding showed negative correlation with duodenal flow of ADF (P = .05).

4.3. Experiment 2.

4.3.1. Animal health. Animals in the second experiment, receiving saponins intraruminally at 4 or 8 % of daily DM intake as a single dose, were affected as indicated by their behavioral changes. These animals showed complete innappetance, appeared lethargic, and lacked rumination behaviour for the duration (4 h) of the motility study.

4.3.2. Motility responses to higher saponin doses. Results of intraruminal administration of saponins in amounts equivalent to 4 and 8 % of DM intake, when the saponins were administered as a single dose rather than in two doses, are shown in Figure 4.8. These tests were not replicated due to the irreversible effects on one of the test animals.

Initial conditions during resting in both sheep indicate normal activity in the forestomach with average reticular and ruminal contraction amplitudes of 40 and 26 mm Hg, frequencies of 1.5 and 2.1 contractions per minute, and durations of 4.0 and 5.6 sec, respectively. In sheep administered saponins at 4 and 8 % of DM intake, significant changes in rumen contraction attributes were apparent in both animals within 15 minutes following dosing, with contractions remaining erratic or undetectable over the following

4 hours. However, changes in the reticulum showed a more gradual response. At 30 min postdosing, administration of 4 and 8 % saponins resulted in reduced contraction frequencies (by 55 and 47 %, respectively) and amplitudes (by 37 and 43 %, respectively). Reticular response to 8 % saponins included further reductions in contraction frequency and amplitude with a 27 min period of complete inactivity at 3 h postdosing.

Beyond this period, motility recordings indicated that reticuloruminal activity in both animals was improving. However, the animal receiving 8 % saponins remained lethargic despite attempts to encourage the animal to eat. Introduction of rumen contents from a healthy animal, intravenous and subcutaneous administration of Lactaid ringers solution, and intraruminal infusion of electrolytic solution were ineffective in stimulating the animal during the following 24 h. Although the animal appeared slightly more alert and body temperature was normal; it refused to stand, eat, or drink during the following 24 h. On the second day following saponin administration, the animal was euthanized by intravenous injection of T-61® (Hoechst, Regina, Sask.). The following autopsy indicated myocardial degeneration and a suspected toxemia related to the changes in the lung. Accumulation of inflammatory cells in the spleen indicated a potential terminal septicemia.

Table 4.1. Composition[a] of hay (dry matter basis)

Item	Period				
	1	2	3	4	SD
Dry matter, %	84.7	85.3	84.9	83.5	.77
Crude protein, %	14.1	13.9	14.7	14.9	.48
Neutral detergent fibre, %	57.7	57.5	56.3	54.2	1.61
Acid detergent fibre, %	35.1	34.7	33.8	32.2	1.28
Acid detergent lignin, %	5.3	5.3	5.3	5.0	.15
Cellulose, %	29.7	29.4	28.4	27.2	1.13
Ash, %	9.3	9.2	10.0	9.9	.41
Gross Energy (Mcal/kg)	4.44	4.42	4.46	4.42	.02

[a]One combined sample of hay was analyzed for each period.

Table 4.2 Composition of saponin extract (dry matter basis)[a]

Item	Amount
Saponin, %	27.8
Sucrose, %	50.6
Ash, %	3.9
Nitrogen, %	1.9
Crude protein, %	.9
Crude fats, %	<.01
Monosaccharides (glucose, xylose, arabinose), %	<.01
Fibre, %	ND[b]
Nucleic acids, %	ND
Energy, (Mcal/kg)	4.89

[a] The extract contained 90.5% DM.
[b] ND, not detectable

Table 4.3 Effect of saponin treatments on time (min) spent resting, ruminating, and feeding during an 8 h recording interval[a] on day 11

		Saponin (% of DM)					P value[c]	
	Day	0	1	2	4	SE[b]	L	Q
Resting	0	233	208	185	187	26.5	.22	.61
	2	180	197	195	235	18.3	.09	.57
	11	205	212	191	229	30.6	.72	.62
Ruminating	0	161	194	196	208	25.9	.27	.68
	2	183	180	179	143	18.9	.20	.41
	11	190	174	210	184	21.3	.87	.80
Feeding	0	86	79	99	85	18.6	.85	.85
	2	117	103	105	102	14.7	.55	.72
	11	85	94	79	67	15.2	.36	.53

[a] 0900 h to 1700 h
[b] Pooled SE (6 df) with four observations per mean.
[c] L = linear effect, Q = quadratic effect.

Table 4.4 Effect of saponin treatments on contraction frequency (contractions/min) of the rumen and reticulum during feeding, ruminating, resting, and total recording period

	Day	Saponin (% of DM)				SE[a]	P value[b]	
		0	1	2	4		L	Q
				RUMEN				
Resting	0	1.73	1.63	1.53	1.74	.11	.90	.19
	2	1.73	1.62	1.73	1.62	.11	.63	.99
	11	1.41	1.40	1.51	1.51	.09	.30	.96
Ruminating	0	1.70	1.76	1.70	1.69	.10	.83	.75
	2	1.64	1.72	1.70	1.70	.07	.65	.58
	11	1.70	1.70	1.52	1.58	.09	.23	.75
Feeding	0	2.85	2.78	2.76	2.53	.19	.31	.69
	2	2.64	2.70	2.38	2.34	.12	.06	.69
	11	2.60	2.60	2.54	2.37	.13	.24	.53
Total 8 h	0	1.91	1.86	1.83	1.86	.09	.67	.63
	2	1.91	1.90	1.87	1.79	.08	.33	.68
	11	1.72	1.74	1.67	1.67	.08	.55	.88
				RETICULUM				
Resting	0	1.12	1.07	1.04	1.06	.05	.39	.50
	2	1.10	1.04	1.07	1.17	.05	.30	.12
	11	0.99	1.00	1.04	1.07	.02	.12	.90
Ruminating	0	1.18	1.22	1.22	1.09	.04	.22	.10
	2	1.14	1.21	1.20	1.22	.03	.10	.33
	11	1.19	1.26	1.16	1.19	.02	.35	.52
Feeding	0	1.74	1.76	1.74	1.55	.05	.03	.06
	2	1.64	1.58	1.60	1.44	.07	.11	.50
	11	1.71	1.63	1.61	1.56	.06	.14	.82
Total 8 h	0	1.25	1.22	1.24	1.16	.03	.10	.32
	2	1.25	1.22	1.26	1.24	.03	.89	.88
	11	1.19	1.21	1.18	1.17	.03	.62	.68

[a] Pooled SE (6 df) with four observations per mean.
[b] L = linear effect, Q = quadratic effect.

Table 4.5 Effect of saponin treatments on contraction amplitude (mm Hg) of the rumen and reticulum during feeding, ruminating, resting, and total recording period

		Saponin (% of DM)					P value[b]	
	Day	0	1	2	4	SE[a]	L	Q
				RUMEN				
Resting	0	21.1	26.1	31.4	16.3	6.28	.75	.16
	2	23.0	19.1	18.7	21.1	4.58	.77	.51
	11	17.0	23.9	26.7	30.5	4.11	.06	.72
Ruminating	0	19.1	16.6	21.0	18.2	4.11	.80	.70
	2	25.7	21.0	25.7	19.6	6.60	.60	.94
	11	21.5	19.9	19.6	27.1	4.10	.41	.31
Feeding	0	23.5	19.5	24.9	17.0	2.55	.26	.47
	2	27.8	22.4	20.4	24.6	5.61	.66	.43
	11	15.5	26.3	27.2	31.3	4.48	.05	.48
Total 8 h	0	20.3	21.3	24.7	17.3	3.4	.73	.27
	2	25.1	20.55	20.7	21.3	5.3	.65	.64
	11	19.2	23.5	23.3	29.3	3.2	.08	.80
				RETICULUM				
Resting	0	38.4	63.9	64.9	46.9	9.0	.53	.05
	2	47.5	49.3	37.1	53.1	9.4	.92	.48
	11	49.3	61.3	34.1	52.5	12.9	.77	.81
Ruminating	0	34.4	46.6	54.0	49.8	10.9	.31	.48
	2	49.2	43.5	34.4	46.3	8.9	.67	.36
	11	50.5	40.6	37.4	48.2	10.2	.83	.35
Feeding	0	35.5	62.2	51.4	52.3	10.0	.41	.25
	2	48.7	44.3	33.0	39.9	6.4	.24	.41
	11	46.1	53.8	36.8	55.9	10.8	.81	.61
Total 8 h	0	36.2	56.2	58.1	48.9	9.7	.39	.18
	2	48.5	46.1	35.3	48.4	8.5	.78	.40
	11	49.3	54.1	36.5	51.4	11.5	.83	.67

[a] Pooled SE (6 df) with four observations per mean.
[b] L = linear effect, Q = quadratic effect.

Table 4.6 Effect of saponin treatments on contraction duration (seconds) of the rumen and reticulum during feeding, ruminating, and resting

	Day	Saponin (% of DM) 0	1	2	4	SE[a]	P value[b] L	Q
				RUMEN				
Resting	0	5.38	5.70	5.55	5.20	.23	.53	.19
	2	5.18	5.15	5.10	5.20	.24	.98	.80
	11	5.10	5.55	5.10	5.28	.43	.97	.76
Ruminating	0	5.23	5.73	5.48	5.00	.16	.24	.02
	2	5.48	5.10	4.65	5.20	.32	.41	.20
	11	5.00	5.63	5.05	5.38	.18	.53	.45
Feeding	0	5.53	5.40	5.90	5.43	.20	.83	.42
	2	5.73	5.10	5.33	5.18	.17	.11	.21
	11	5.45	5.68	5.13	5.50	.36	.81	.84
Total 8 h	0	5.35	5.66	5.56	5.16	.15	.35	.77
	2	5.44	5.12	4.94	5.19	.22	.34	.34
	11	5.11	5.57	5.10	5.30	.24	.92	.30
				RETICULUM				
Resting	0	4.38	4.35	4.55	4.70	.28	.39	.77
	2	4.48	4.20	4.18	4.48	.20	.55	.34
	11	4.38	4.15	4.25	4.20	.08	.27	.30
Ruminating	0	4.08	4.00	4.08	4.13	.10	.65	.57
	2	4.13	4.05	4.18	4.13	.12	.82	.92
	11	3.78	3.93	3.83	3.83	.11	.92	.52
Feeding	0	4.38	4.28	4.08	4.33	.14	.41	.96
	2	4.25	4.08	4.08	4.33	.14	.73	.18
	11	4.30	4.25	4.15	4.10	.07	.06	1.00
Total 8 h	0	4.28	4.17	4.36	4.43	.17	.43	.62
	2	4.30	4.12	4.14	4.24	.13	.83	.33
	11	4.13	4.08	4.04	4.03	.06	.25	.75

[a] Pooled SE (6 df) with four observations per mean.
[b] L = linear effect, Q = quadratic effect.

Table 4.7 Effects of saponin treatments on digesta flow and passage rates

Item	Saponin (% of DM)					P value[b]	
	0	1	2	4	SE[a]	L	Q
Sheep weight, kg	59.5	59.1	60.9	59.8	.72	.47	.62
PARTICULATES							
Rumen pool size, g	217	239	289	311	49.9	.12	.82
Passage rate, %/h	3.6	3.4	3.0	2.9	.35	.14	.89
Particle outflow, g/day	168	168	175	210	12.3	.05	.20
MRT[c], h	30.4	32.8	39.1	35.6	4.3	.30	.52
Particle DM, % of	1.63	1.67	2.33	2.24	.26	.08	.79
RUMEN FLUID							
Pool size, L	13.9	14.7	13.8	15.0	.66	.49	.77
Passage rate, %/h	4.6	4.4	4.7	4.4	.17	.79	.84
Passage rate, L/day	15.2	15.3	15.4	15.4	.35	.65	.91
MRT, h	22.4	23	21.8	22.9	1.01	.96	.79

[a] Pooled SE (6 df) with four observations per mean.
[b] L = linear effect, Q = quadratic effect.
[c] mean retention time

Table 4.8 Effect of saponin treatments on ruminal and total digesta flows and digestibilities

	Saponin (% of DM)					P value[b]	
	0	1	2	4	SE[a]	L	Q
INTAKE							
DM, g/d	1185	1138	1185	1117	22.0	.17	0.66
OM, g/d	1071	1029	1071	1010	19.8	.17	.66
NDF, g/d	668	642	668	631	12.2	.17	.68
ADF, g/d	402	386	402	380	7.26	.17	.68
N, g/d	27.3	26.2	27.3	25.7	.52	.16	.65
GE, Mcal/d	5.25	5.04	5.25	4.95	.10	.17	.66
FLOW TO DUODENUM, g/d							
OM	318	358	411	380	17.8	.02	0.1
NDF	160	169	195	180	9.46	.09	.25
ADF	99.8	104	119	111	6.02	.12	.33
Total N	14.4	17.4	20.0	18.1	.85	.01	.02
Bacterial N	5.65	7.42	10.96	8.83	1.70	.13	.29
Other N	8.82	10.00	9.12	9.22	1.22	.97	.66
BACTERIAL EFFICIENCIES, g bacterial N/kg OM apparently digested							
Apparent	7.8	11.5	18.9	14.7	3.5	.12	.29
RUMINAL APPARENT DIGESTION, %							
OM	70.2	65.4	61.6	62.1	1.90	.02	.13
NDF	76.0	73.9	70.6	71.4	1.58	.05	.41
ADF	75.1	73.3	70.1	70.7	1.69	.07	.51
N	47.3	34	27	29.5	3.03	.001	.04
TOTAL APPARENT DIGESTION, %							
OM	77.5	76.3	76.6	73.9	1.07	.13	.42
NDF	75.1	73.4	74.1	71.7	1.28	.15	.77
ADF	73.4	71.7	72.8	70.2	1.41	.22	.75
N	78.4	77.8	78.5	74.4	1.08	.06	.16

[a] Pooled SE (6 df) with four observations per mean.
[b] L = linear effect, Q = quadratic effect.

Table 4.9 Effects of saponin treatments on rumen fluid VFA concentration (mM), ruminal pH, and ruminal protozoal populations (x10^5/mL).

Item	Dose					P value[b]	
	0	1	2	4	SE[a]	L	Q
Volatile fatty acids			DAY 0				
Total	70.5	64.6	71.7	66.6	2.49	.68	.87
Acetic	51.4	46.2	51.3	47.2	1.61	.31	.73
Propionic	12.9	11.7	13.5	12.9	.61	.52	.61
Isobutyric	.51	.61	.63	.60	.04	.17	.17
Butyric	4.60	4.90	5.00	4.70	.34	.82	.34
Isovaleric	.45	.54	.63	.61	.06	.07	.32
Valeric	.62	.57	.64	.62	.06	.78	.79
Caproic	.08	.07	.08	.08	.007	.04	.49
pH	6.65	6.77	6.66	6.71	.07	.83	.69
Protozoa	1.68	1.87	1.56	1.56	.20	.48	.66
Volatile fatty acids			DAY 2				
Total	75.5	73.4	68.9	97.0	2.9	.004	.002
Acetic	52.8	50.4	47.4	68.1	2.2	.005	.002
Propionic	14.7	15.0	14.5	19.6	.73	.005	.02
Isobutyric	.40	.46	.27	.19	.06	.02	.27
Butyric	6.4	6.4	5.7	7.7	.34	.07	.02
Isovaleric	.37	.43	.24	.21	.06	.05	.46
Valeric	.67	.69	.70	1.03	.08	.02	.10
Caproic	.10	.08	.08	.13	.02	.37	.12
pH	6.44	6.54	6.39	5.97	.06	.002	.006
Protozoa	1.63	1.10	.30	.12	.28	.005	.56
Volatile fatty acids			DAY 14				
Total	63.3	66.8	64.8	74.0	7.3	.40	.71
Acetic	43.8	45.6	44.4	51.3	5.0	.38	.63
Propionic	12.4	14.3	14.0	14.3	1.7	.52	.67
Isobutyric	.46	.41	.25	.28	.06	.04	.64
Butyric	5.5	5.4	5.2	7.0	.55	.13	.15
Isovaleric	.54	.44	.29	.29	.06	.01	.44
Valeric	.56	.63	.66	.79	.08	.09	.73
Caproic	.08	.05	.01	.11	.01	.58	.004
pH	6.55	6.61	6.56	6.45	0.09	.38	.35
Protozoa	1.65	0.95	0.32	0.19	0.12	.0001	.05

[a]SE values based on 3 observations on day 0; 4 observations on days 2 and 14
[b] L = linear effect, Q = quadratic effect.

Table 4.10 Effects of saponin treatments on blood hematocrit values

	Saponin (% of DM)					P value[b]	
Day	0	1	2	4	SE[a]	L	Q
0	29.5	32.3	29.8	31.3	1.4	.68	.68
2	31.3	31.5	30.5	32.8	.6	.21	.12
14	30.0	33.0	30.5	30.3	.9	.67	.11

[a]SE values based four observations.
[b] L = linear effect, Q = quadratic effect.

Table 4.11 Effects of saponin treatments on oxygen consumption, carbon dioxide and methane production, and energy losses in methane and heat[a].

Item	Saponin (% of DM)					P value[c]	
	0.00	1	2	4	SE[b]	L	Q
OXYGEN CONSUMPTION							
L/day	563	576	562	540	26.5	.51	.53
L/day per kg body wt.	9.19	9.62	9.16	9.09	.48	.72	.62
CO_2 AND CH_4 PRODUCTION							
CO_2, L/day	527	530	602	543	35.6	.48	.42
CH_4, L/day	28.7	30.5	30.2	28.2	3.0	.91	.54
CO_2, L/day per kg body wt.	8.00	8.83	9.81	9.12	.59	.39	.47
CH_4, L/day per kg body wt.	.48	.50	.49	.48	.04	.99	.56
ENERGY LOSSES							
Heat production[d], Mcal/day	2.79	2.85	2.88	2.72	.13	.77	.45
Methane, Mcal/day	.27	.29	.29	.27	.03	.91	.54

[a] Based on 24 h averages on day 12.
[b] SE values based 4 observations.
[c] L = linear effect, Q = quadratic effect.
[d] calculated with the McLean equation (1972).

Table 4.12. Correlation between total observed digestibilities and frequency (contractions per min), amplitude (mm Hg), and duration (s) of reticular and ruminal contractions on day 11

Item	Frequency				Amplitude				Duration			
	Rest[a]	Rum[a]	Feed[a]	8 h	Rest	Rum	Feed	8 h	Rest	Rum	Feed	8 h
					RETICULAR vs RUMINAL CONTRACTIONS							
	.90[b]	.74	.83	.87	-.04	-.28	-.02	-.10	-.03	.11	.36	.04
	.0001[c]	.001	.0001	.0001	.88	.30	.93	.72	.90	.68	.17	.86
					RETICULAR CONTRACTIONS							
OM	.59	.45	.62	.56	-.39	-.33	-.19	-.35	-.55	-.22	-.47	-.56
	.02	.08	.01	.02	.14	.21	.47	.18	.03	.40	.06	.02
NDF	.59	.46	.64	.58	-.40	-.34	-.21	-.36	-.55	-.20	-.46	-.55
	.02	.07	.01	.02	.13	.19	.43	.17	.03	.45	.07	.03
ADF	.59	.48	.66	.59	-.40	-.35	-.23	-.37	-.55	-.19	-.47	-.54
	.02	.06	.01	.02	.12	.19	.39	.17	.03	.49	.07	.03
Total N	.59	.39	.59	.53	-.38	-.34	-.18	-.35	-.56	-.25	-.50	-.58
	.02	.13	.02	.03	.15	.19	.51	.19	.02	.34	.05	.02
					RUMINAL CONTRACTIONS							
OM	.30	.47	-.27	.47	.29	.65	.16	.48	.36	.35	-.42	.24
	.26	.07	.31	.07	.28	.006	.55	.06	.17	.18	.11	.37
NDF	.29	.47	-.25	.48	.27	.65	.15	.47	.36	.36	-.40	.25
	.27	.06	.35	.06	.31	.007	.57	.07	.17	.18	.12	.35
ADF	.30	.48	-.24	.50	.25	.63	.13	.44	.38	.36	-.39	.27
	.26	.06	.36	.05	.36	.009	.64	.09	.15	.17	.13	.31
Total N	.30	.43	-.25	.46	.31	.70	.20	.52	.34	.33	-.41	.22
	.25	.10	.35	.08	.24	.003	.46	.04	.19	.21	.11	.41

[a] Rest, Rum, Feed refer to measurements made during the resting, ruminating, and feeding periods

[b] Pearson correlation coefficients (R)

[c] Probability values based on 16 observations

Table 4.13 Correlation between digesta flows and frequency (contractions per min), amplitude (mm Hg), and duration (s) of reticular contractions during resting, ruminating, feeding, and total recording interval on day 11

Item	Frequency				Amplitude				Duration			
	Rest[a]	Rum[a]	Feed[a]	8 h	Rest	Rum	Feed	8 h	Rest	Rum	Feed	8 h
DUODENAL FLOW, g/d												
DM	.57[b]	.69	.69	.64	-.12	.03	-.10	-.07	-.42	.09	-.34	-.32
	.02[c]	.003	.003	.007	.67	.92	.70	.81	.10	.75	.20	.23
OM	.59	.65	.68	.64	-.16	-.05	-.13	-.12	-.49	.06	-.39	-.38
	.02	.01	.004	.008	.55	.85	.64	.66	.05	.83	.13	.15
NDF	.41	.42	.55	.45	-.33	-.36	-.21	-.32	-.61	-.10	-.42	-.52
	.12	.11	.03	.08	.22	.17	.44	.22	.01	.70	.11	.04
ADF	.31	.34	.50	.37	-.35	-.41	-.19	-.55	-.61	-.17	-.38	-.55
	.24	.20	.05	.16	.19	.11	.47	.03	.01	.54	.14	.03
Total	.65	.75	.71	.71	-.09	.07	-.09	-.04	-.40	.07	-.35	-.31
	.01	.001	.002	.002	.74	.81	.74	.87	.12	.78	.19	.24
Bacterial N	.79	.71	.71	.76	-.11	.01	-.15	-.08	-.44	.06	-.37	-.33
	.0002	.002	.002	.001	.69	.96	.58	.76	.08	.83	.16	.21
Other N	-.33	.09	-.02	-.13	.04	.12	.14	.09	.10	.04	.05	-.33
	.22	.74	.94	.63	.89	.65	.61	.74	.71	.88	.86	.21
BACTERIAL EFFICIENCY, g/kg feed												
Apparent	-.50	-.60	-.50	.73	-.19	-.40	-.08	-.10	.24	-.37	.33	-0.4
	.05	.01	.05	.001	.48	.12	.77	.73	.36	.16	.21	.19

[a] Rest, Rum, Feed refer to measurements made during the resting, ruminating, and feeding periods

[b] Pearson correlation coefficients (R)

[c] Probability values based on 16 observations

Table 4.14. Correlation between digesta flows and frequency (contractions per min), amplitude (mm Hg), and duration (s) of ruminal contractions during resting, ruminating, feeding and total recording interval on day 11

Item	Frequency				Amplitude				Duration			
	Rest[a]	Rum[a]	Feed[a]	8 h	Rest	Rum	Feed	8 h	Rest	Rum	Feed	8 h
DUODENAL FLOW, g/d												
DM	.54[b]	.30	.67	.48	.09	.04	-.04	.02	.21	-.10	-.20	.08
	.03[c]	.26	.004	.06	.74	.88	.88	.93	.43	.73	.36	.78
OM	.59	.29	.66	.50	.18	.14	.02	.12	.19	-.10	-.30	.04
	.02	.28	.01	.05	.50	.62	.94	.66	.48	.68	.24	.89
NDF	.51	.20	.53	.40	.32	.33	.04	.28	.06	-.20	-.50	-.14
	.05	.46	.04	.12	.22	.21	.87	.29	.82	.40	.07	.60
ADF	.42	.13	.45	.31	.33	.37	.02	.30	.01	-.20	-.50	-.19
	.11	.63	.08	.24	.21	.16	.95	.26	.97	.40	.05	.49
Total N	.61	.40	.69	.57	.06	.004	-.01	-.004	.33	-.10	-.20	.18
	.01	.12	.003	.02	.84	.99	.96	.99	.21	.72	.48	.51
Bacterial	.72	.41	.61	.63	.07	.09	.04	.05	.32	-.01	-.40	.16
	.002	.12	.01	.01	.79	.73	.88	.85	.22	.97	.13	.54
Other N	-.20	-.01	.16	-.13	-.03	-.20	-.10	-.13	.01	-.20	.46	-.02
	.37	.97	.55	.64	.90	.45	.64	.64	.97	.46	.08	.93
BACTERIAL EFFICIENCY, g/kg feed												
Apparent	.65	.30	.54	.55	.08	.07	.05	.04	.33	.14	-.33	.23
	.006	.25	.03	.03	.78	.80	.86	.88	.22	.61	.22	.40

[a] Rest, Rum, Feed refer to measurements made during the resting, ruminating, and feeding periods

[b] Pearson correlation coefficients (R)

[c] Probability values based on 16 observations

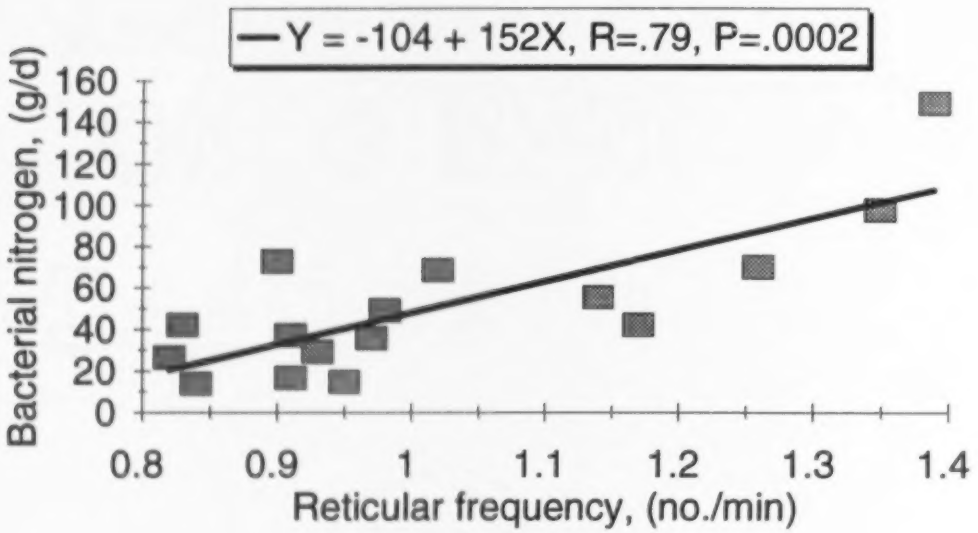


Fig. 4.1. Relationship between reticular frequency during resting on day 11 and duodenal bacterial nitrogen flow (N=16).

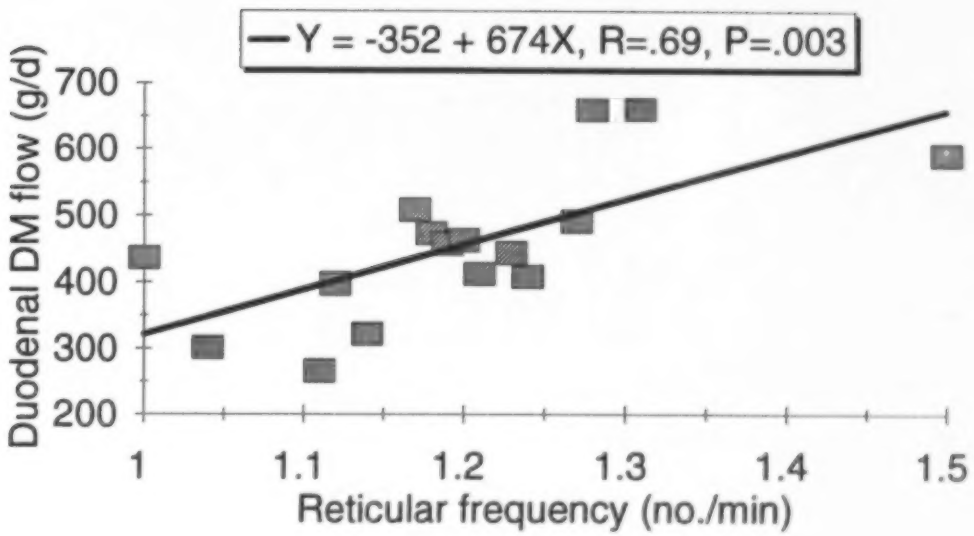


Fig. 4.2. Relationship between reticular frequency during rumination on day 11 and duodenal digesta flow (N=16).

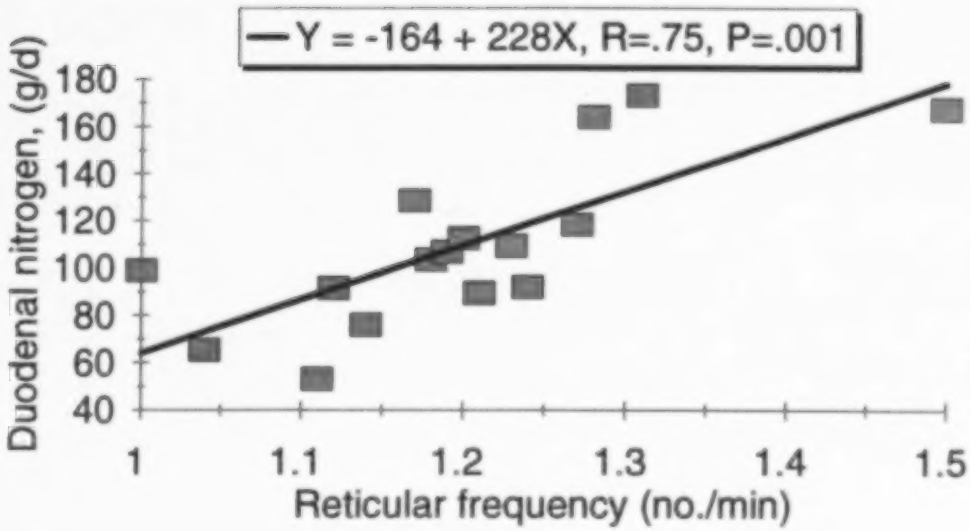


Fig. 4.3. Relationship between reticular frequency during rumination on day 11 and duodenal nitrogen flow (N=16).

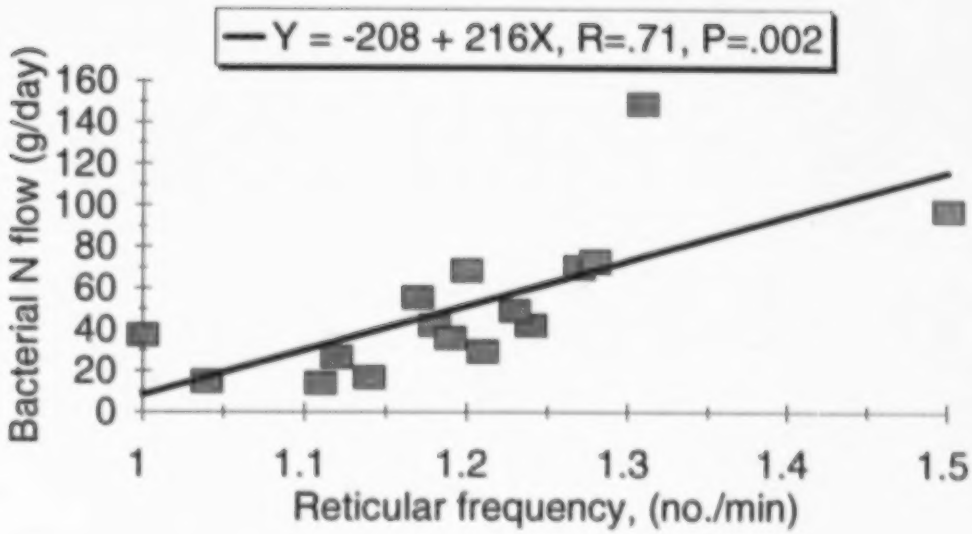


Fig. 4.4. Relationship between reticular frequency during rumination on day 11 and duodenal bacterial N flow (N=16).

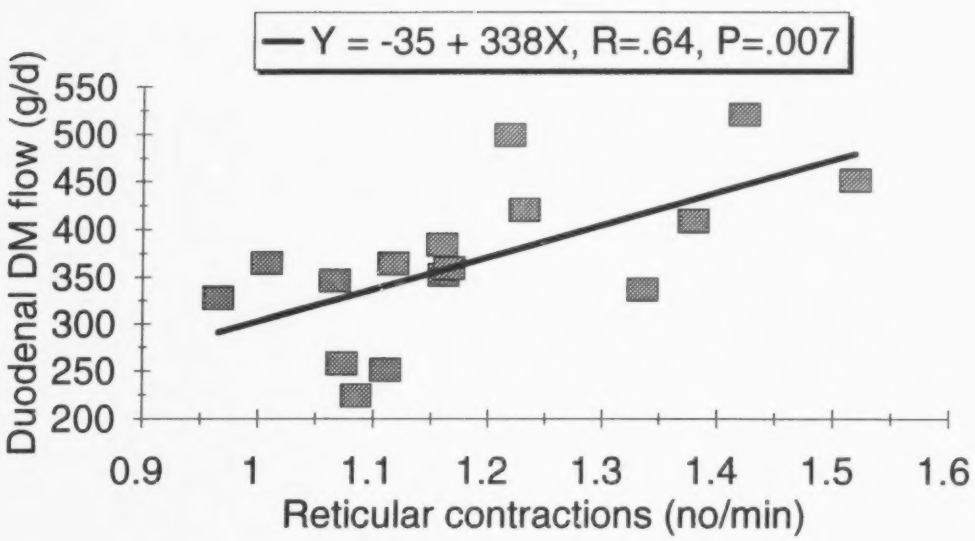


Fig. 4.5. Relationship between total reticular frequency recording on day 11 and duodenal digesta flow (R=16).

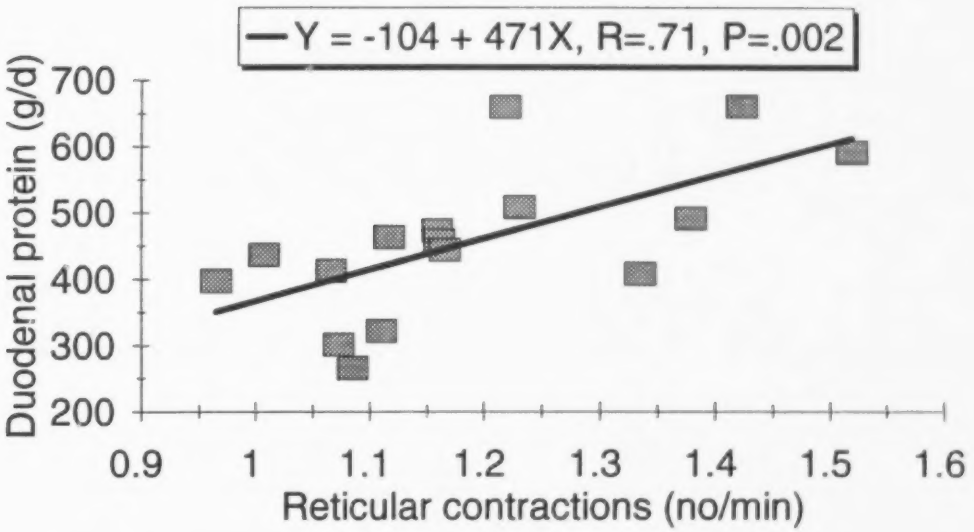


Fig. 4.6. Relationship between total reticular frequency on day 11 and duodenal protein flow (N=16).

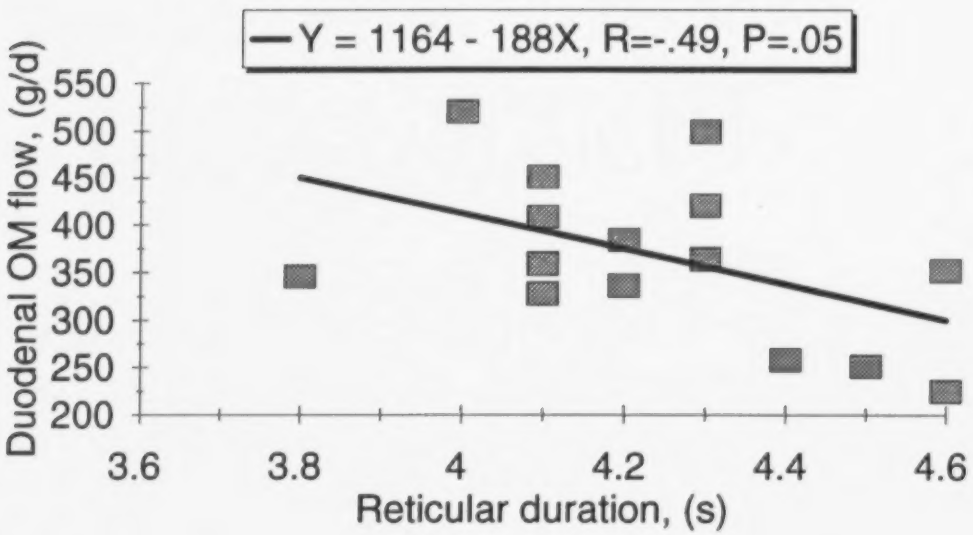


Fig. 4.7. Relationship between reticular duration during resting on day 11 and duodenal organic matter flow (R=16).

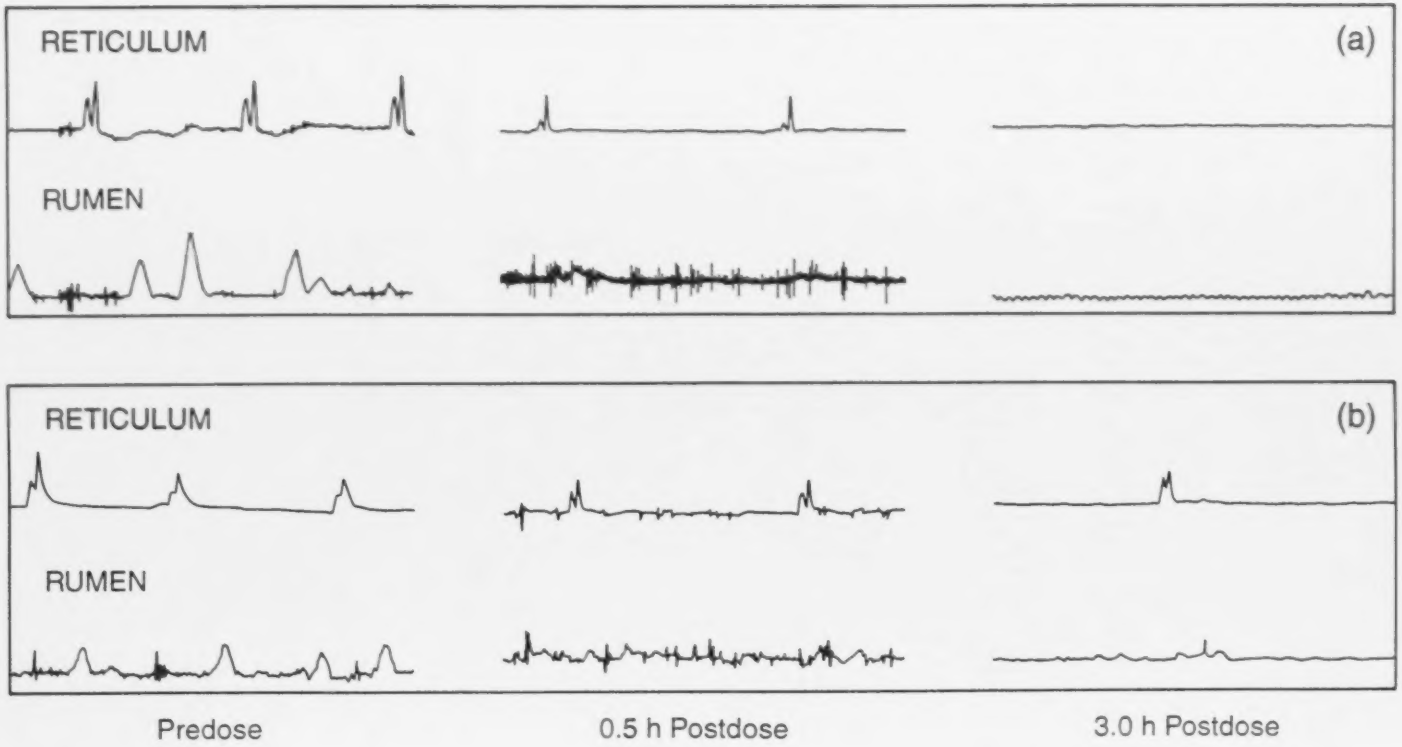


Figure 4.8. Chart recordings of 2 minute intervals of reticular and ruminal contractions in sheep prior to, .5 h after, and 3 h after dosing with saponins at 8 % (a) and 4 % (b) of DM intake.

Chapter 5. Discussion

5.1. Alfalfa saponins.

Saponins commonly found in alfalfa are primarily oleanane triterpenoids (Bondi et al., 1973). Although similar compounds are found in a wide variety of plants (Simes, 1950; Eade et al., 1963; Decosterd et al., 1987; Higuchi et al., 1988; Slacanin, 1988), slight differences in structure have been shown to alter biological activities considerably (Zimmer er al., 1967; Livingston et al., 1977). Therefore, it was important to obtain saponins directly from alfalfa for this experiment. The composition and variety of saponins in alfalfa will vary to some degree (Table 2.1), however Oleszek (1991) demonstrated that the most abundant saponin in the alfalfa cultivar studied was structurally identical in both the leaf and root samples. Since saponins are generally more concentrated in roots (Shany et al., 1972; Fenwick and Oakenfull, 1983), alfalfa roots were selected as an available and economically feasible saponin source. Also, use of alfalfa roots as the saponin source was beneficial because it precluded the need for ether extraction in removal of plant pigments; which is commonly used when extracting plant leaf material (Shany et al., 1970a; Gestetner et al., 1971).

Published extraction procedures for saponins incorporating solvent extraction (ethanol/methanol/water) followed by isolation by acid hydrolysis (Shany et al., 1970a) or cholesterol precipitation (Lindahl et al., 1954) were not considered for this study since the resulting sapogenins have been shown to exhibit greater biological activity than their respective saponins (Glauret et al., 1962; Zimmer et al., 1977). Procedures allowing

isolation of small amounts of highly purified saponins (Oleszek et al., 1990; Massiot et al., 1991) were not feasible for isolation of saponins in quantities suitable for animal feeding trials.

Analysis of the saponin extract by the haemolytic micromethod (Jurzysta, 1979) which indicated that saponins were present at 27.8 % of the extract DM (Table 4.2), was based on comparison with a commercial saponin standard since alfalfa saponin standards were unavailable. Although commercially available saponins (from *Quillaja saponaria*) do possess triterpenoidal saponins with structural similarity to alfalfa saponins (Higuchi et al., 1988), the biological activity of these two saponin sources may differ.

Although the primary components of the saponin extract have been identified (Table 4.2), approximately 16 % of the contents are unknown. Analysis of the extract for crude fats, other sugars, fibre, and nucleic acids did not identify any other significant fractions. However, it appears that some nitrogen-containing organic compounds other than amino acids must be present. It is possible that unidentified compounds in the extract may have had some influence on the animals.

5.2. Amounts of added saponins.

Saponin levels, for the first part of this experiment, were selected to represent the range of saponin concentrations normally encountered in forages. The typical saponin concentrations in North American alfalfa varieties is approximately 2-3 % (Gestetner et al., 1970; Shany et al., 1970a). Although alfalfa forage saponin levels vary between .1 and 8 % of plant DM, levels can reach 5.6 % in mature plants (Fenwick and Oakenfull,

1983) and 8 % in alfalfa sprouts (Livingstone et al, 1979). The occurrence of saponins in alfalfa forage in concentrations of greater than 2-3 % is not uncommon (Pedersen et al., 1967). Therefore, saponin additions of up to 4 % of the mixed hay DM represent levels which may be encountered by livestock. Since animals were kept at a low level of feeding (maintenance DM intake of 2 % body wt; fed semi-continuously at 4 h intervals), but could consume levels of 3-5% body weight, total daily saponin intake could potentially be much higher than those encountered in this experiment, especially if high-saponin alfalfa is consumed. Also, because saponins are readily water soluble (Morris et al., 1961) and located in peripheral plant surfaces (Simes, 1950; Pedersen, 1975; Quazi, 1975) they are likely to be quickly released into the rumen contents. Therefore, saponins could be readily available in the rumen liquid to exhibit their actions soon after the ingestion of feed. For this reason, during the second experiment, administration of saponins at 8 % of the DM intake at maintenance in a single dose was selected to represent the potential upper limit of total saponin intake in animals fed alfalfa ad libitum. This higher level, or administration as a single daily dose, would likely have been too severe to allow a long term study.

Coarsely chopped mixed grass hay was selected for this experiment specifically as a low-saponin feed. The only grass currently known to contain saponins is oats (*Avena sativa*), in which only the steroidal aglycones have been identified at total levels up to .1 % (Fenwick and Oakenfull, 1983). There has been no identification of saponins in other grasses. Also, this feed was selected to provide strong stimulation for reticuloruminal contractions to aid in motility measurements.

5.3. Animal behaviour responses to saponins.

Animals in exp. 1, receiving total saponins up to 4 % DM intake in two intraruminal doses daily, did not demonstrate significant changes in behaviour during the experiment. However, animals receiving the highest saponin level in the first study spent 30 % more time resting and subsequently less time ruminating and eating, than the control animals on day 2. This could indicate a behavioral change in response to saponins, although it is not supported statistically (Table 4.3).

In contrast, complete lack of rumination behaviour was observed when saponins were administered as single doses at levels of 4 and 8 % DM intake in exp 2. In this situation, animals were observed as lethargic with complete disinterest in feeding for several hours following saponin administration. These results support data reported by Lindahl et al. (1957).

Previous research on alfalfa saponins had indicated that these compounds are (Lindahl et al., 1957), or could be (Lu and Jorgenson, 1987) involved in ruminant bloat; while others have discredited this idea (Majak et al., 1980). Although the current study was not directed towards observing bloat, nor was this condition observed; there was a visible increase in leakage of rumen fluid around the ruminal cannulae of animals receiving the highest level of saponins which may be an indicaiton of greater froth formation, although frothiness of rumen fluid was not examinaed in this study. This leakage could have precluded the abnormal distention of the rumen typically associated with bloat, and appears to have influenced the marker estimations of duodenal and total DM flow.

5.4. Motility responses to saponins.

Alfalfa saponins at 4 % and 8 % of DM intake, administered as single doses (exp 2), eliminated ruminal contractions within minutes and suppressed activity for several hours (Figure 4.1). Reticular contractions were significantly reduced in frequency, amplitude, and duration within 30 minutes following administration of saponins and were eliminated completely; following administration of the 8 % saponin level, for a 27 minute period at 3 hours postdosing. These results support the observations of Lindahl et al. (1957) that alfalfa saponins inhibit forestomach motility and eructation. In this study, improved reticular motility 4 hours after administration of saponins indicated that the animal was either adapting to this acute dietary change, rumen microbes were digesting the compounds (Gutierrez et al., 1958), or saponins were diluted to levels that reduced their effect. Lindahl et al. (1957) also reported that effects of saponins on motility and eructation appear greatest immediately after dosing, either intraruminally or intravenously. Subsequently, animals either showed no changes, an initial response with recovery within several hours, or an immediate response with persistent effects sometimes resulting in fatality; depending upon the quantities of saponins administered.

Adaptation to the dietary changes within a matter of hours could partially explain the apparent limited treatment response in motility, which was observed from recordings on days 2 and 11 (in exp 1). The tendency towards reduced frequency of contractions during feeding, with increased saponins administered, in the rumen ($P = .06$) and reticulum ($P = .11$) on day 2 suggests that the immediate impact of saponins, as observed from acute administration of higher saponin levels, continued to have a measurable effect

on motility on day 2. Although no significant effects on frequency were noted on day 11, ruminal contraction amplitudes showed increases during resting (P = .06) and feeding (P = .05) of 79 and 102 % respectively. Increased ruminal contraction amplitudes may have been a response to the increased ruminal DM pool size rather than a direct effect of saponins. It may be of importance that the trends were more significant during feeding since this is a time when contraction frequency is greatest and a response more likely to be demonstrated. Continuous introduction of saponins to the diet does not appear to have had any adverse effects on amplitude (Table 4.5) and duration (Table 4.6) of reticular contractions.

Although no mechanism of action has yet been determined for the inhibitory effects of saponins on smooth muscle, it is possible that epithelial receptors in the luminal epithelium are involved. It is however, more likely a result of the impact of saponins on biological membranes. Triterpenoid saponins have a specific affinity for cholesterol, causing disruption of cell membranes as noted in blood haemolysis, fungal growth inhibition, and insect toxicity. Studies indicate that saponins alter membrane permeability in the small intestine to facilitate uptake of materials to which the gut would normally be impermeable (Johnson et al., 1986). If such an imbalance occurred, in the reticuloruminal epithelium, chemoreceptors involved in regulation of motility could be affected and respond by inhibiting motility.

Since "saponins fail to cross the gut and enter the blood stream" (Small, et al., 1990), and responses to the compounds are immediate, as indicated by this study and the work of Lindahl (1957), the site of action must lie within the reticulorumen. Yet,

evidence that saponins and sapogenins are more highly active when administered intravenously than orally (Lindhal, 1957; Lu and Jorgenson, 1982), also suggests that receptors may exist which respond directly to humoral saponin levels or secondarily to changes influenced by them.

During motility recordings, reticular and ruminal contraction frequencies (Table 4.4) were highest during feeding, intermediate during rumination, and lowest during resting; supporting results already presented for the reticulum of cattle (Ulyatt et al., 1984; Ruckebusch, 1989; Okine and Mathison, 1991; Okine et al., 1993). Differences observed between ruminal and reticular contraction frequencies are explained by the secondary ruminal contractions associated with eructation as previously described by Ruckebusch (1989). Reticular contraction amplitudes were greater than corresponding ruminal contraction amplitudes for any given activity, supporting previous reports (Phillipson, 1970; Okine et al., 1993). However, indications that reticular contraction amplitudes in cattle during resting are greater than those during feeding or ruminating (Okine et al., 1993) were not present in this study. Ruminal contraction duration was consistently greater than reticular contraction duration for all activities during the motility recordings. Reticular contraction duration was greatest during resting, intermediate during feeding, and least during rumination which supports evidence previously reported for cattle (Okine et al., 1993).

5.5. Effect of saponins on digesta flow.

The studies by Lindahl et al. (1957) suggested that inhibition of forestomach motility by saponins, which would be expected to severely affect digesta flow, was a

major factor predisposing ruminant animals to bloat. Previous reports have suggested that bloat-susceptible animals exhibit a lower fractional outflow rate (FOR) of reticulorumen digesta than do animals considered bloat tolerant (Majak et al., 1986; Okine et al., 1989a). The current study did not result in significant differences in the rumen fluid dilution rate between sheep given different amounts of saponins, although there was a 19 % reduction (P = .14) in the particulate passage rate (Table 4.7). It would have been of interest to know how liquid dilution rate and particle FOR were affected when animals were first exposed to saponins.

Total duodenal flow was significantly increased (P = .02) by increasing amounts of administered saponins as observed from digesta samples collected at the end of the experimental periods on days 13 to 14 (Table 4.8). Similar results were previously reported by Lu and Jorgensen (1987) as feed digestibilities were reduced with higher levels of administered saponins. They further noted that the effect was greater on a concentrate than a roughage diet, with significant increases in total digestibility on the concentrate diet. This study indicated an overall decrease in digestibility of the roughage diet which could be due to differences in feed used.

Estimation of digesta passage rates by digesta markers (Table 4.7) also indicated a 25 % increase (P = .05) of particle outflow per day which supports the observations of Lu and Jorgensen (1987). The greater ruminal amplitudes (P = .05) seen on day 11 may have influenced the increase in flow of feed particles. Increased duodenal OM flow (P = .02) is also in agreement with the report of Lu and Jorgensen (1987). The mordanted fiber used in this study was less than 1 mm in length. Therefore, we must consider that

increased particulate passage rates could be an indicator of small particle movement rather than total digesta DM flow.

Relationships between reticuloruminal motility and digesta flow in animals were observed, with reticular contraction frequencies explaining the variation in duodenal digesta flow (Table 4.12) and digestibilities (Table 4.13) to a greater extent than contraction duration. Contraction amplitudes did not show any relationship to digesta flow. The positive correlation between reticuloruminal frequency and digesta flow observed in this study supports observations by Okine et al. (1989b, 1993) and Okine and Mathison (1991). However, reduced duodenal digesta flow in response to increased reticular contraction duration during resting (Table 4.12) is in contrast with results of Okine and Mathison (1991) observed in cattle at a restricted feed intake.

5.6. Microbial populations.

Reduced protozoal populations on days 2 and 14 with increasing doses of saponins agree with previous reports (Lu and Jorgensen, 1987; Lu et al., 1987). A reduced protozoal population may explain the 132 % increase in duodenal bacterial DM and N flows in the duodenum on days 13 and 14 in response to increasing saponin doses since protozoal predation on bacteria would have been highly reduced. The significance of bacterial engulfment by rumen ciliate protozoa has been well documented (Coleman and Reynolds, 1982; Williams and Coleman, 1989), with evidence that protozoa were responsible for 88 % of bacterial protein turnover (Wallace and McPherson, 1987). Rumen ciliate protozoa obtain amino acids for growth principally by the engulfment and

entrapment of rumen bacteria (Coleman, 1980). Defaunation of the rumen has led to four-fold increases in total bacterial population in the rumen of sheep (Newbold and Hillman, 1990), suggesting that bacterial increases observed in this study with reductions in the protozoal population are to be expected. Presence of cholesterol in eukaryotic (including protozoa) cell membranes, but not in prokaryotic bacterial cells, suggests selective susceptibility of rumen ciliate protozoans to saponins.

Reduced protozoal populations on day 14 (Table 4.9) indicate that the effects of saponins on the microbial populations are sustained, in contrast with the effect on motility. Observations that average protozoal populations decreased and bacterial production increased over the duration of the experiment (data shown in Appendix) suggests that the 7 day adjustment periods may have been too short to allow for re-establishment of initial microbial populations.

5.7. Rumen pH and VFA.

Decreased (P = .002) pH and increased (P = .004) VFA concentrations on day 2, in response to increasing saponins, would be due to an inhibition of VFA absorption by the ruminal epithelium, reduced outflow from the reticulorumen, or increased microbial VFA production. The effects of saponins on cell membranes could have caused initial impairment of VFA absorption on day 2. Effects of saponins on lower gut absorption has been demonstrated by reductions of carrier-mediated transport mechansims (Johnson et al., 1986). Although reduced protozoal populations would significantly reduce the predation of bacteria and allow their population to increase, it is likely that bacterial

activity could account for the sharp increase in VFA concentration observed as early as day 2. This is particularly true considering that conditions on day 14 also showed suppressed protozoal populations and increased bacterial production (P = .13), but no related differences in VFA concentration in response to increased saponins. Lack of treatment differences in VFA concentration on day 14 could be explained by an increased efficiency of rumen microbial cells (P = .02). Previous reports indicate that as microbial growth efficiency (measured as g drycells per mole ATP) increases, the percentage of the fermented organic matter that is partitioned into VFA's and gases (methane and carbon dioxide) decreases while the amount entering into microbial cells increases (Leng, 1982a,b).

5.8. Energy partitioning.

The lack of differences in oxygen consumption and carbon dioxide and methane production are likely due to adaptation as these observations were taken on day 12. However, it appears that an increase in methane production may have been observed during initial administration of saponins since methane production has been shown to be inversely related to passage rates (Okine et al., 1989a). Since there was an apparent adaptation in reticuloruminal motility and no observable differences in animal activity, we would not have expected any influence on heat production in the current study.

5.9. Relationship between reticular motility and flow.

Reticular frequencies did not appear to be infuenced by administered saponins on

day 11 (Table 4.4), suggesting adaptation to saponins by contraction frequency. Yet there is a significant positive correlation between reticular contraction frequencies and digesta flows, when examined over all treatments (Table 4.13), supporting previous reports by Okine et al., (1989a).

5.10. Overview

Contraction frequencies during feeding tended to decrease, in the rumen (P = .06) and reticulum (P = .11) on day 2 in response to added saponins. Long term administration (11 d) of saponins indicated an adaptation in factors controlling contraction frequency, but rumen amplitudes were increased during resting (P = .06) and feeding (P = .05) 79 and 102 %, respectively as the level of saponins increased from 0 to 4 %. Saponins caused increases in volatile fatty acid concentrations (P = .004) and lowered pH (P = .002) after 2 d of administration, however, had no effect on day 11. On day 11, protozoal populations decreased (P = .005) and bacterial production linearly increased (P = .01) in response to higher levels of saponins. Apparent digestibilities of OM were reduced by 12 % in both the rumen (P = .02) and total digestive tract (P = .06) with increasing levels of saponins. Increasing saponin levels also resulted in increased duodenal flows of OM, NDF, and total N (P = .02, .09, and .01, respectively). Following administration of higher saponin concentrations (as 4 and 8 % DM intake in a single dose) ruminal contractions were suppressed within 15 minutes and reticular contractions notably decreased at 30 min postdosing in both animals. At 3 h following saponin administration, the animal receiving an 8 % dose exhibited no forestomach motility for

a 27 min period. Both animals failed to display feeding or rumination behavior for several hours following saponin administration.

5.11. Implications.

It was concluded that normal levels of saponins in alfalfa could have a significant initial impact on ruminoreticular motility with adaptation of factors controlling frequency while rumen contraction amplitudes actually increased following long term administration of higher levels of saponins. The long term effects of alfalfa saponins included a decrease in protozoal populations, increased bacterial N flow, and reduced ruminal and total tract digestibilities.

Chapter 6. General Discussion and Conclusions

6.1. Contributions of this study.

Previous studies on forestomach motility have been limited to a collection of responses to acute intravenous or oral administration of alfalfa extracted saponins (Lindahl et al., 1957). This study therefore provides the first longer term study concerning the influence of alfalfa saponins on reticuloruminal motility. The current study provided evidence that alfalfa saponins, at levels considered typical of alfalfa forage, can significantly inhibit reticuloruminal motility. However, analysis of contraction parameters suggested that continuous administration of saponins led to an adaptation of those factors controlling forestomach contraction frequency. Rumen contraction amplitudes increased significantly with increased levels of saponins, although the reason for and effect of this situation is not known. The effects of alfalfa saponins on forestomach motility are significant and this indicates that alfalfa varieties of higher saponin content could have detrimental effects on the grazing animal, suggesting that the bloat potential of saponins still exists and must be considered when selecting grazing alfalfa as a management option.

Secondly, this study indicated that increasing levels of alfalfa saponins reduced apparent ruminal and total tract digestibilities. A reduction in ruminal digestibilities by 12 % ($P = .02$) is in agreement with the results of Lu and Jorgensen (1987) who reported an 11 % decrease ($P = .04$) in digestibility on roughage diets with 4 % saponins added. They also reported a similar, although greater effect with a concentrate diet. This study also indicated that increasing levels of saponins tended to decrease total tract OM

digestibility (P = .06). Lu and Jorgensen reported that total tract digestibility with roughages was not affected by saponin administration but was increased with a concentrate diet. The reduction in ruminal digestibilities may play a significant role in terms of increasing the levels of bypass protein at the duodenum, however the potential advantages of this effect may be negated by decreased whole tract digestibility.

Thirdly, this study indicated that continued administration of saponins has a sustained impact on microbial populations. The reduction of protozoal populations in response to increasing levels of administered saponins supports previous reports (Lu, 1982; Lu and Jorgenson, 1987), which have reported decreases of 34 and 66 % in protozoal populations in response to administration of 2 and 4 % alfalfa saponins respectively. The increased amount of bacterial protein flowing through the duodenum daily, following long term administration of alfalfa saponins reported in this study, contrasts with the results of Lu and Jorgensen (1987) who found that bacterial N flow to the duodenum was reduced by 20 and 30 % with saponins added at levels of 2 and 4 % respectively. However, the increase in bacterial N flow to the duodenum reported in this study would support the evidence that suppressed protozoal populations allow an increase in bacterial population, due to limited competition as previously reported (Newbold and Hillman, 1990).

Finally, this study indicated that saponins at levels naturally occurring in alfalfa, did not influence the total energy expenditure as heat production or the energy lost as methane (d 12). However, it would be interesting to determine these effects immediately following administration of saponins to a non-adapted animal, since increases in methane

production would be expected as a result of reduced digesta flow at that time. However, as a natural component in feed, alfalfa saponins do not appear to alter the animals energy expenditure.

6.2. Considerations for future research

There is little doubt that alfalfa saponins possess significant biological activities. It is important that we understand these properties more completely, and the nutritional implications of saponins as forage components. We would benefit from understanding the antinutritional factors of saponins and their potential as natural plant products for use in manipulating ruminant digestion. Further, there are indications that saponins have no detrimental effects in primates, but rather may be beneficial in human medicine; especially for those who suffer from elevated blood cholesterol levels (Malinow et al., 1978).

Before further animal studies can be conducted, it is important that we establish a quantitative HPLC procedure for the analysis of alfalfa saponins. Up to now, quantification of alfalfa saponins has been dependent on non-specific tests including fungistatic and haemolytic assays. Although these assays have been beneficial in estimating total saponin levels, the results can be highly variable with limited confidence in their accuracy. A recently published HPLC procedure (Nowacka and Oleszek, 1994), specific to alfalfa saponins, using external standards of purified alfalfa saponins to quantify saponins in the plant extracts, shows promise for routine analysis of forage samples. This technique could assist us in determining saponin profiles from forage

samples and allow us to distinguish between saponins known to posses biological activity from those having little or no activity. There has been evidence that saponin composition varies between species, geographical location, and with stages of plant development (Pedersen, 1967; Quazi, 1975). Using this HPLC method would provide information on contents and variations of saponins in North American alfalfa varieties. Further information could be gained from following changes in saponin profiles with stages of plant development and responses to environmental factors to determine not only the changes of total saponin concentrations, but identify changes of levels of the biologically active components.

Although we understand that alfalfa saponins possess a wide variety of biological activities including altered absorption of nutrients in the GI tract and inhibition of smooth muscle activity, the specific mechanisms of action still elude us. Further investigations into these activities would provide us with a better understanding of these compounds and allow us to determine their potential negative impacts as feed components or their beneficial uses depending on the application. Reports have indicated that alfalfa saponins have specific activities on altered absorption of nutrients (Johnson et al., 1986) and effects on transmural potential differences in mammalian small intestines (Oleszek et al., 1994b). These studies could provide us with the background to an understanding of specific mechanisms of actions of saponins.

The effect of alfalfa saponins could be more fully understood by determining their rate of release from plant material and net accumulation within the rumen environment. Although we have evidence that alfalfa saponins are concentrated in plant surface tissues

(Pedersen, 1975; Quazi, 1976) and >90 % can removed by a single extraction (Nowacka and Oleszek, 1994), there has been no report of their behavior within the rumen. If there is a high rate of release as expected, this would allow saponins to exhibit their actions relatively soon after ingestion of feed. Evidence suggests that alfalfa saponins can be degraded by rumen bacteria (Gutierrez and Davis, 1962) although there has been no report of their relative efficiency. The effect of microbial degradation could significantly affect the net accumulation of saponins in the rumen. Complete and rapid degradation of saponins could reduce the potential negative effects of saponins, while partial degradation of saponins could form compounds with greater biological activity (Oleszek et al., 1992). Therefore, it is important that we study and understand the role of microbial degradation on the influence of alfalfa saponins in the ruminant animal.

In conclusion, this research and that of Lu and Jorgensen (1987) indicates that alfalfa saponins are a worthy area of study in spite of the suggestion by Majak et al. (1980) that alfalfa saponins were not related to bloat potential. It is hoped that the results of this study will stimulate further investigation into the abundence and activities of alfalfa saponins and re-evaluate their potential role as a bloat inducing agent.

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Chapter 8. Appendices

Appendix I: Haemolytic Assay.

An isotonic buffered solution is prepared by dissolving 3.95 g Na_2HPO_4, 0.76 g KH_2PO_4, and 7.2 g NaCl per L volume of water and adjusting pH to 7.0. Gelatin (3.0 g) was dissolved in isotonic buffered saline (35 mL), mixed with 8 mL of blood, and stirred and heated to dissolve the gelatin completely. The solution was set in a cool water bath to bring the temperature down to 20°C before application to glass plates. Glass plates (20 x 20 cm) were prepared by positioning them on a smooth surface and restricting their movement. Precooling the plates with an ice covered tray ensured quicker setting of the gelatin and prevented spillover of the blood solution. With a thin chromatography spreader (Desaga, Heidelberg, Germany) set to a thickness of 0.5 mm, the cooled blood/gelatin suspension was poured into the spreader and spread with a single smooth movement over at least two complete glass plates. Two extra glass plates were used as the starting and stopping points for the spreader. The gelatin in the solution maintained stability of the suspension at room temperature and allowed uninhibited diffusion of saponins. Once set, these blood plates were ready for the application of test solutions.

Solid aniline blue dye was added to the isotonic buffered solution at a concentration of 2.5 mg/mL to provide clear resolution of the zones of haemolysis in the standards and samples. Purified saponins from Mathison/Coleman/Bell (MCB) and Sigma were each prepared at concentrations of 0, 0.5, 1, 1.5, 2, 2.5, 3, 5, 10, and 20 mg/mL in the aniline blue isotonic buffered saline solution for use as the saponin standards. The saponin extract to be tested was prepared in a similar manner to provide a concentration

of 20 mg/mL for testing. Each standard and saponin extract was applied as 10 µL drops with at least ten replications. Prepared plates were set in a humid sealed container to prevent drying and allowed to incubate for 20 hours at room temperature before measuring haemolytic rings. Glass plates were removed from the container, dried at room temperature, and haemolytic ring diameters measured when the glass plates were set on an overhead projector that provided a magnification of 7.8 x. Linear standard curves were obtained for the MCB and Sigma standards.

Appendix II: Transducer calibrations.

Pressure transducers were calibrated at three levels, representing 10, 20, and 40 mm Hg prior to each measurement period. Calibrations involved the injection of air from a syringe into a closed system with a mercury manometer for calibration. The manometer was maintained at the level of the sensor in the transducer. This closed system made calibration relatively easy since slowly removing the syringe allowed the system to consistently and quickly return to atmospheric conditions, considered to exert 0 mm Hg pressure on the transducer.

Appendix III: Atomic Absorption.

The procedure used for analysis of cobalt and chromium in digesta samples was a modification of a procedure described by Reese et. al. (1994). All digesta samples were dried at 60°C to a constant weight and ground with a Wiley mill using a 1 mm screen.

Due to the small sample sizes available, especially for duodenal digesta, duplicate subsamples of approximately 200 mg were digested rather than the suggested subsamples of .25 g, .5 g, and 1 g. The majority of the duodenal samples for determination of particulate dilution rates and ruminal particulate volume were approximately 1-2 g dry matter, making replication of samples possible only in smaller quantities. The procedure of Reese et. al. (1994) had been chosen since the complexation with EDTA did provide absorption values consistently 15-20% greater (personal observations/unpublished results) than previous methods which relied on simple acid digestion of samples.

Duodenal samples in 0.2 g duplicates and faecal samples as 0.25, 0.5, and 1.0 g subsamples were weighed onto weighing paper and transferred to tared 50-mL Erlenmeyer flasks, and the flasks were reweighed. For matrix free standard flasks, marker-free samples (taken prior to dosing) were used. Matrix standards were prepared by the addition of 2.5 mL of 4-N HNO_3 containing appropriate elements in amounts calculated to provide standards with final concentrations of 0, 0.5, 1, 2, 4, and 8 µg/mL in the final 30 mL solutions. A standard set was made for each concentration of feces. Standards were then reweighed to determine the precise volume of standard concentrate added by dividing the increased weight by the predetermined specific gravity of the standard solutions. Ten mL of 4-N HNO_3 was added to each sample and blank standard flask (zero ppm), and 7.5 mL to each matrix standard flask. After allowing all samples to 'wet' in the initial volume of 4-N HNO_3, a second 10 mL addition of acid was used to wash down any sample residues adhering to the sides of the flask and bring the total liquid volume to 20 mL per flask. Unstoppered flasks were set in the storage containers

(up to 75 per container) and placed in a 70°C oven. To each storage container, approximately 100 g of sodium bicarbonate was added to absorb acid fumes and neutralize any potential spills. Water added to the storage container to the liquid level within the flasks acted as a water bath to provide even heat distribution around the flasks and also retards the evaporation of the sample solutions. Samples were digested for 18-24 hours per 0.25 g of material, with attention given to maintaining the water level in the storage container and agitation of the flasks twice daily to ensure complete digestion. After digestion, water was siphoned from the containers to provide stability when the flasks were removed from the oven. Acid digested samples contained little fibrous material and had a pale yellow appearance. These samples were allowed to cool to room temperature before the addition of 10 mL of Reagent B (16 g EDTA, 5 g $LiOH.H_20$, 400 mL water, brought to volume of 1L with addition of NH_4OH). Addition of the NH_4OH solution caused an exothermic reaction and turned solutions brown (darker colours formed with greater amounts of sample digested). Samples were once again placed in the storage containers as before and incubated in the oven for a further 18-24 hours. After removing the flasks from the oven, the outer surface of the flasks were rinsed under a stream of hot water to wash off any residues of sodium bicarbonate and flasks were set on paper towel covered trays to dry completely in the fume hood as they returned to room temperature. All flasks were reweighed for determination of liquid volumes. In general, solution volumes were approximately 28 mL after partial evaporation. Once weighed, each sample was filtered through quantitative filter paper directly into appropriately labelled containers (Nalgene centrifuge tubes) large enough to hold the total liquid volume. Filtered samples

were then ready for measurement on the Perkin-Elmer 4000 atomic absorption spectrometer (Perkin-Elmer, Norwalk, CT). Equipment was zeroed with distilled deionized water, and analyzed for cobalt and chromium with an air/acetylene flame at 240.7 nm and 357.9 nm respectively. Duodenal samples were collected prior to and 2, 4, 8, 12, 18, 24, 30, 32, 48, 56, and 72 hours post dosing. Particulate dilution rates from the duodenal samples were analyzed through linear regression analysis of the natural log values of chromium concentrations on a DM basis, and particulate DM volumes determined by extrapolation of the regression equation to determine chromium concentration at time zero.

Appendix IV: High Performance Liquid Chromatography of DAPA.

Standard samples contained 3 mL of 6 M hydrochloric acid, 100 µL 6.4 µmol/mL DAPA, and 100 µL 3.2 µmol/mL internal standard. To the cooled hydrolyzed samples was added 100 µL water and 100 µL of 3.2 µmol/mL internal standard (aminocaprylic acid). Hydrolysates and standards were centrifuged for 15 minutes at 2500 *g*. Vials (1.5 mL) were prepared with 100 µL of standard/sample hydrolysate, 100 µL of 5.63 M NaOH, 200 µL of saturated $K_2B_4O_7.4H_2O$, and 1000 µL HPLC grade water. To complete the analysis of DAPA, 50 µL of OPA reagent solution was added and mixed with 75 µL of prepared hydrolysate or standard. A 15 µL volume of derivatized sample was then injected and subjected to HPLC.

A binary gradient changing from a polar to a non-polar solvent was used for sample elution. The polar solvent consisted of a water-methanol mixture (60:40, v/v)

containing 0.1M sodium acetate and 7.5 mM HTMA. The non-polar solvent consisted of a methanol-water mixture (95:5, v/v) containing 7.5 mM HTMA. Solvents were adjusted to pH 6.4 with glacial acetic acid. Under these conditions DL-a-aminocaprylic acid, DD,LL-DAPA, and DL-DAPA were found to elute at 28.4, 33.4, and 35.3 minutes respectively.

Appendix V: Duodenal cannulation.

Feed is withdrawn 48 h and water 24 h before surgery. Antibiotics, 10 x 10^6 IU procaine penicillin G is injected intramuscularly twice daily starting 24 h prior to surgery and continuing for 10 days after. Induction of anaesthesia is intramuscular Atravet and intravenous Thiopentone sodium. Anaesthesia is maintained with halothane and oxygen delivered through a semi-closed system with intermittent positive pressure ventilation. Sheep are in left lateral recumbency for surgery.

The right side of the abdomen is clipped, scrubbed and draped by a standard four-corner draping technique. The body wall is opened as a 20 cm curved skin incision made parallel and 4 cm ventral to the costal arch from the level of the transverse plane of rib 13 to rib 8. Subcutaneous tissue, cutaneous trunci muscle, and the external layer of the rectus sheath are incised in the same plane. Transverse abdominal muscle is incised dorsoventrally in the same direction as its muscle fibres at the level of the transverse plane of the 10th intercostal space from the costal arch to the border of the superficial epigastric vein. This incision is continued through the internal layer of the rectus sheath and the peritoneum.

Upon entry to the abdomen, the pylorus and proximal 20 cm of the cranial part of the duodenum are exteriorized and separated from the remainder of the incision by moist towels. Site of cannula insertion is centred approximately 8 to 10 cm distal to the pylorus for double T cannulae. Omentum is elevated from the duodenum for a distance corresponding to the length of the cannula barrel. Care is taken to preserve major branches of duodenal vessels and, thus, to minimize effects of cannulation on blood flow to the area. A 5 to 6 cm enterotomy is made along the right side of the duodenum in the area previously freed of omentum. Intestinal clamps may be applied if accessible. Intermittent suction is required to remove intestinal contents.

The body of the cannula is inserted into the intestinal lumen. The enterotomy is closed adjacent to the barrel of the cannula with 00 nonabsorbable polybutilate suture (Ethibond, Pitman-Moore and Co., Washington Crossing, N.J.) in a simple interrupted pattern. The cut edges to the enterotomy are inverted, and two purse string sutures of the same type of suture material are placed snugly around the cannula barrel. The surgical field is lavaged with sterile saline and the inner collar is inserted over the cannula barrel for designs that included a collar.

In preparation for cannula exteriorization a 2 cm diameter piece of skin is removed from the right body wall in the 10th intercostal space at the level of frontal plane of the shoulder joint. By sharp and blunt dissection, muscle layers and peritoneum are incised. The cannula barrel is pushed through the body wall at this site, and the outer collar is inserted to hold the cannula in place.

The incision is closed in the following manner. Peritoneum and transverse

abdominal muscle fascia are closed in a simple continuous pattern with Number 2 chromic surgical gut suture. The muscle belly of the rectus abdominus is apposed in a simple interrupted pattern with the same suture material. The external layer of the rectus sheath is sutured in a simple interrupted pattern with Number 2 polyglycolic acid suture. The cutaneous trunci muscle is closed in a simple continuous pattern with Number 1 chromic surgical gut suture. Skin is sutured by a simple interrupted pattern with Number 2 monofilament nylon.

Sheep are hospitalized until they recover adequately from the surgical procedure (5 to 10 days).

Table 7.1. Effect of saponin treatments on contraction frequency (contractions min^{-1}) in the rumen and reticulum during feeding, ruminating, and resting

		Saponin (% of DM)					Probability		
	Day	0	1	2	4	SE[a]	P[b]	A[b]	D[b]
					RUMEN				
Resting	0	1.73	1.63	1.53	1.74	.11	.70	.02	.20
	2	1.73	1.62	1.73	1.62	.11	.32	.05	.79
	11	1.41	1.40	1.51	1.51	.09	.36	.01	.68
Ruminating	0	1.70	1.76	1.70	1.69	.10	.76	.04	.94
	2	1.64	1.72	1.70	1.70	.07	.27	.01	.88
	11	1.70	1.70	1.52	1.58	.09	.57	.23	.46
Feeding	0	2.85	2.78	2.76	2.53	.19	.53	.46	.69
	2	2.64	2.70	2.38	2.34	.12	.09	.12	.18
	11	2.60	2.60	2.54	2.37	.13	.04	.09	.58
					RETICULUM				
Resting	0	1.12	1.07	1.04	1.06	.05	.88	.03	.71
	2	1.10	1.04	1.07	1.17	.05	.08	.04	.30
	11	0.99	1.00	1.04	1.07	.02	.04	.00	.41
Ruminating	0	1.18	1.22	1.22	1.09	.04	.58	.01	.23
	2	1.14	1.21	1.20	1.22	.03	.04	.00	.23
	11	1.19	1.26	1.16	1.19	.02	.01	.00	.11
Feeding	0	1.74	1.76	1.74	1.55	.05	.03	.02	.05
	2	1.64	1.58	1.60	1.44	.07	.05	.08	.27
	11	1.71	1.63	1.61	1.56	.06	.09	.00	.44

[a] Pooled SE (6 df) with four observations per mean.
[b] P=period, A=animal, D=dose.

Table 7.2. Effect of saponin treatments on contraction amplitude (mm Hg) in the rumen and reticulum during feeding, ruminating, and resting

	Day	Saponin (% of DM) 0	1	2	4	SE[a]	Probability P[b]	A[b]	D[b]
				RUMEN					
Resting	0	21.1	26.1	31.4	16.3	6.3	.29	.28	.43
	2	23.0	19.1	18.7	21.1	4.6	.17	.25	.90
	11	17.0	23.9	26.7	30.5	1.1	.05	.09	.23
Ruminating	0	19.1	16.6	21.0	18.2	4.1	.53	.30	.63
	2	25.7	21.0	25.7	19.6	6.6	.64	.53	.92
	11	21.5	19.9	19.6	27.1	4.1	.09	.15	.58
Feeding	0	23.5	19.5	24.9	17.0	2.55	.11	.06	.21
	2	27.8	22.4	20.4	24.6	5.61	.19	.24	.81
	11	15.5	26.3	27.2	31.3	4.48	.06	.15	.18
				RETICULUM					
Resting	0	38.4	63.9	64.9	46.9	9.0	.11	.88	.20
	2	47.5	49.3	37.1	53.1	9.4	.24	.17	.68
	11	49.3	61.3	34.1	52.5	12.9	.30	.59	.55
Ruminating	0	34.4	46.6	54.0	49.8	10.9	.30	.53	.64
	2	49.2	43.5	34.4	46.3	8.9	.23	.15	.68
	11	50.5	40.6	37.4	48.2	10.2	.09	.63	.78
Feeding	0	35.5	62.2	51.4	52.3	10.0	.26	.32	.38
	2	48.7	44.3	33	39.9	6.4	.03	.10	.42
	11	46.1	53.8	36.8	55.9	10.8	.17	.60	.60

[a] Pooled SE (6 df) with four observations per mean.
[b] P=period, A=animal, D=dose.

Table 7.3. Effect of saponin treatments on contraction duration (seconds) in the rumen and reticulum during feeding, ruminating, and resting

		Saponin (% of DM)					Probability		
	Day	0	1	2	4	SE[a]	P[b]	A[b]	D[b]
					RUMEN				
Resting	0	5.4	5.7	5.5	5.2	.23	.39	.17	.49
	2	5.2	5.2	5.1	5.2	.24	.17	.67	.99
	11	5.1	5.5	5.1	5.3	.43	.67	.24	.86
Ruminating	0	5.2	5.7	5.5	5.0	.16	.10	.05	.07
	2	5.5	5.1	4.7	5.2	.32	.69	.49	.41
	11	5.0	5.6	5.0	5.4	.18	.32	.21	.15
Feeding	0	5.5	5.4	5.9	5.4	.20	.28	.03	.35
	2	5.7	5.1	5.3	5.2	.17	.44	.14	.14
	11	5.5	5.7	5.1	5.5	.36	.52	.29	.75
					RETICULUM				
Resting	0	4.4	4.3	4.5	4.7	.28	.12	.41	.80
	2	4.5	4.2	4.2	4.5	.20	.68	.98	.70
	11	4.4	4.2	4.3	4.2	.08	.27	.06	.29
Ruminating	0	4.1	4.0	4.1	4.1	.10	.01	.21	.86
	2	4.1	4.0	4.2	4.1	.12	.03	.16	.89
	11	3.8	3.9	3.8	3.8	.11	.24	.55	.81
Feeding	0	4.4	4.3	4.1	4.3	.14	.45	.48	.53
	2	4.3	4.1	4.1	4.3	.14	.45	.48	.53
	11	4.3	4.3	4.2	4.1	.07	.24	.11	.24

[a] Pooled SE (6 df) with four observations per mean.
[b] P=period, A=animal, D=dose.

Table 7.4. Effect of saponin treatments on contraction frequency (contractions per 8 h) in the rumen and reticulum during feeding, ruminating, and resting

	Day	Saponin (% of DM) 0	1	2	4	SE[a]	P-value[b] L	Q
				RUMEN				
Resting	0	1208	951	836	962	148	.24	.24
	2	941	1038	993	1003	141	.83	.77
	11	907	936	887	1007	109	.62	.69
Ruminating	0	816	1052	1033	1092	140	.24	.56
	2	891	829	878	883	111	.96	.78
	11	888	808	935	842	92	.98	.95
Feeding	0	740	700	739	620	141	.63	.79
	2	912	869	842	706	113	.25	.69
	11	891	829	878	883	111	.26	.70
				RETICULUM				
Resting	0	809	653	560	595	103	.16	.39
	2	596	604	625	813	70	.08	.25
	11	611	641	596	703	89	.58	.68
Ruminating	0	556	692	716	683	96	.38	.41
	2	622	676	658	527	81	.44	.30
	11	682	659	748	659	76	.96	.68
Feeding	0	432	408	516	392	87	.98	.59
	2	577	476	525	444	82	.38	.91
	11	416	448	257	328	63	.25	.65

[a] Pooled SE (6 df) with four observations per mean.
[b] L = linear effect, Q = quadratic effect.

Table 7.5. Effect of saponin treatments on contraction amplitude (cm Hg $8h^{-1}$) in the rumen and reticulum during feeding, ruminating, and resting

	Day	Saponin (% of DM) 0	1	2	4	SE[a]	P-value[b] L	Q
				RUMEN				
Resting	0	2780	2018	2152	1645	411	.13	.76
	2	2558	2090	2015	2606	576	.98	.39
	11	1281	2327	2745	3261	526	.04	.63
Ruminating	0	1502	1899	2690	1976	524	.38	.33
	2	2417	1790	2096	1698	763	.61	.89
	11	2033	1469	1849	2376	507	.56	.32
Feeding	0	1568	1858	3022	1018	102	.91	.31
	2	2129	1735	1683	1461	584	.46	.89
	11	1196	1791	1520	1841	453	.44	.77
				RETICULUM				
Resting	0	280	400	266	298	70.1	.81	.55
	2	273	277	228	334	56.3	.61	.40
	11	292	367	216	410	91.2	.64	.54
Ruminating	0	193	317	384	316	66.1	.19	.20
	2	316	290	223	224	57.5	.23	.82
	11	351	248	301	284	55.3	.58	.47
Feeding	0	163	263	380	189	95.4	.66	.18
	2	248	219	197	245	46.5	.89	.44
	11	189	273	114	127	39.2	.10	.40

[a] Pooled SE (6 df) with four observations per mean.
[b] L = linear effect, Q = quadratic effect.

Table 7.6. Effect of saponin treatments on contraction duration (min per 8 h) in the rumen and reticulum during feeding, ruminating, and resting

	Day	Saponin (% of DM) 0	1	2	4	SE[a]	P-value[b] L	Q
				RUMEN				
Resting	0	108.0	90.0	76.2	82.0	14.2	.20	.44
	2	82.0	89.7	82.5	86.0	12.1	.91	.88
	11	71.3	87.8	74.8	86.5	13.0	.81	.96
Ruminating	0	70.0	101.0	96.0	90.2	11.9	.33	.17
	2	82.0	69.0	69.5	76.7	13.1	.80	.47
	11	73.5	76.5	79.3	76.5	9.0	.78	.76
Feeding	0	68.5	63.7	70.2	56.5	14.1	.66	.76
	2	86.2	74.7	73.2	60.2	9.3	.11	.94
	11	62.8	72.0	50.0	51.5	11.2	.31	.74
				RETICULUM				
Resting	0	59.3	47.3	42.0	46.3	6.2	.16	.24
	2	44.5	42.5	43.5	58.3	5.7	.15	.19
	11	44.3	44.0	41.8	49.3	6.3	.67	.56
Ruminating	0	37.8	45.5	49.3	47.0	7.0	.36	.50
	2	43.3	46.0	46.0	35.8	6.2	.45	.33
	11	43.0	43.0	47.8	41.8	4.8	.96	.56
Feeding	0	31.8	29.3	39.5	29.5	6.4	.91	.58
	2	41.0	32.5	35.3	32.3	5.8	.40	.65
	11	30.0	31.8	24.8	22.0	4.2	.15	.61

[a] Pooled SE (6 df) with four observations per mean.
[b] L = linear effect, Q = quadratic effect.

Table 7.7. Effect of saponin treatments on contraction frequency (as a fraction of day 0) in the rumen and reticulum during feeding, ruminating, and resting

		Saponin (% of DM)					P-value[b]	
	Day	0	1	2	4	SE[a]	L	Q
				RUMEN				
Resting	2	0.99	0.99	1.14	0.95	0.06	0.95	0.16
	11	0.82	0.86	0.98	0.89	0.07	0.31	0.41
Ruminating	2	0.97	0.97	1.01	1.01	0.07	0.63	0.97
	11	1.04	0.96	0.90	0.95	0.10	0.49	0.56
Feeding	2	0.93	0.99	0.87	0.93	0.08	0.7	0.99
	11	0.91	0.95	0.93	0.94	0.07	0.89	0.87
				RETICULUM				
Resting	2	0.99	0.97	1.05	1.11	0.03	0.02	0.23
	11	0.89	0.93	1.00	1.00	0.06	0.19	0.74
Ruminating	2	0.98	1.00	0.99	1.12	0.05	0.1	0.3
	11	1.02	1.04	0.95	1.10	0.04	0.43	0.16
Feeding	2	0.95	0.89	0.92	0.94	0.05	0.91	0.48
	11	0.98	0.92	0.92	1.01	0.03	0.61	0.05

[a] Pooled SE (6 df) with four observations per mean.
[b] L = linear effect, Q = quadratic effect.

Table 7.8. Effect of saponin treatments on contraction amplitude (as a fraction of day 0) in the rumen and reticulum during feeding, ruminating, and resting

		Saponin (% of DM)					P-value[b]	
	Day	0	1	2	4	SE[a]	L	Q
				RUMEN				
Resting	2	1.10	0.76	0.91	1.33	0.22	0.44	0.14
	11	0.87	1.18	1.27	1.96	0.24	0.02	0.46
Ruminating	2	1.46	1.59	1.13	1.17	0.57	0.62	0.94
	11	1.19	1.51	0.92	1.59	0.38	0.73	0.66
Feeding	2	1.14	1.46	0.83	1.38	0.30	0.96	0.72
	11	0.81	1.86	1.06	1.98	0.59	0.35	0.92
				RETICULUM				
Resting	2	1.21	0.87	0.56	1.31	0.35	0.99	0.17
	11	1.30	1.10	0.54	1.22	0.28	0.55	0.16
Ruminating	2	1.44	1.03	0.82	1.13	0.38	0.53	0.38
	11	1.47	0.90	0.87	1.09	0.30	0.41	0.23
Feeding	2	1.43	0.73	0.77	0.98	0.36	0.45	0.26
	11	1.37	0.89	0.84	1.41	0.30	0.97	0.13

[a] Pooled SE (6 df) with four observations per mean.
[b] L = linear effect, Q = quadratic effect.

Table 7.9. Effect of saponin treatments on contraction duration (as a fraction of day 0) in the rumen and reticulum during feeding, ruminating, and resting

		Saponin (% of DM)					P-value[b]	
	Day	0	1	2	4	SE[a]	L	Q
				RUMEN				
Resting	2	0.97	0.91	0.92	1.01	0.05	0.57	0.23
	11	0.95	0.97	0.93	1.03	0.07	0.53	0.54
Ruminating	2	1.07	0.89	0.85	1.04	0.07	0.74	0.04
	11	0.96	0.98	0.93	1.08	0.05	0.23	0.24
Feeding	2	1.04	0.95	0.92	0.96	0.05	0.27	0.27
	11	0.99	1.06	0.88	1.02	0.08	0.83	0.72
				RETICULUM				
Resting	2	1.02	0.97	0.93	0.96	0.06	0.48	0.51
	11	1.00	0.96	0.95	0.93	0.05	0.4	0.78
Ruminating	2	1.02	1.02	1.03	1.01	0.03	0.96	0.72
	11	0.93	0.98	0.95	0.94	0.03	0.96	0.38
Feeding	2	0.97	0.95	0.90	0.96	0.06	0.72	0.52
	11	0.98	1.00	0.92	0.91	0.04	0.14	0.8

[a] Pooled SE (6 df) with four observations per mean.
[b] L = linear effect, Q = quadratic effect.

Table 7.10 Acute effects of alfalfa saponins on forestomach motility during resting[a]

Time	Frequency (#/min)		Amplitude (mmHg)		Duration (s)	
(h)	4%	8%	4%	8%	4%	8%
			RUMEN			
Predose	2.20	2.00	25.0	27.8	5.7	5.6
0.5	NA	NA	NA	NA	NA	NA
1.0	NA	NA	NA	NA	NA	NA
1.5	NA	NA	NA	NA	NA	NA
2.0	NA	NA	NA	NA	NA	NA
2.5	NA	NA	NA	NA	NA	NA
3.0	NA	NA	NA	NA	NA	NA
3.5	NA	NA	NA	NA	NA	NA
			RETICULUM			
Predose	1.55	1.33	37.5	43.8	4.0	4.0
0.5	0.70	0.70	23.8	25.0	3.5	3.5
1.0	0.70	0.70	25.0	47.5	3.6	4.0
1.5	0.60	0.60	23.8	22.5	3.7	3.5
2.0	0.70	0.50	20.0	21.8	3.5	3.8
2.5	0.60	0.20	20.0	12.5	3.5	3.5
3.0	0.60	NA*	23.8	NA*	3.6	NA*
3.5	0.60	0.40	25.0	12.5	3.8	3.7

[a]Animals exhibited only resting behavior following dosing of saponins.
NA = no measurable contractions.
*Complete lack of activity observed for over 27 minutes.

Appendix XVI Experimental data

Column	Entry
1	PERIOD
2	ANIMAL
3	DOSE
4	mm WOOL HINDQUARTER
5	mm WOOL BACK
6	mm WOOL FORELEG
7	mm WOOL AVERAGE
8	DAY 0 HEMATOCRIT
9	DAY 2 HEMATOCRIT
10	DAY 14 HEMATOCRIT
11	AVERAGE HEMATOCRIT
12	DAY 0 RUMEN pH
13	DAY 2 RUMEN pH
14	DAY 14 RUMEN pH
15	AVERAGE pH
16	kg ANIMAL WEIGHT
17	O2 CONSUMPTION (ml/min)
18	FREQ-RET-REST-D0-#
19	FREQ-RET-REST-D0-MIN
20	FREQ-RET-REST-D0- no/mi
21	FREQ-RET-REST-D2-#
22	FREQ-RET-REST-D2-MIN
23	FREQ-RET-REST-D2- no/mi
24	FREQ-RET-REST-D11-#
25	FREQ-RET-REST-D11-MIN
26	FREQ-RET-REST-D11- no/m
27	FREQ-RET-RUM-D0-#
28	FREQ-RET-RUM-D0-MIN
29	FREQ-RET-RUM-D0- no/min
30	FREQ-RET-RUM-D2-#
31	FREQ-RET-RUM-D2-MIN
32	FREQ-RET-RUM-D2- no/min
33	FREQ-RET-RUM-D11-#
34	FREQ-RET-RUM-D11-MIN
35	FREQ-RET-RUM-D11- no/mi
36	FREQ-RET-FEED-D0-#
37	FREQ-RET-FEED-D0-MIN
38	FREQ-RET-FEED-D0-no/min
39	FREQ-RET-FEED-D2-#
40	FREQ-RET-FEED-D2-MIN
41	FREQ-RET-FEED-D2-no/min
42	FREQ-RET-FEED-D11-#
43	FREQ-RET-FEED-D11-MIN
44	FREQ-RET-FEED-D11-no/mi
45	FREQ-RUM-REST-D0-#
46	FREQ-RUM-REST-D0-MIN
47	FREQ-RUM-REST-D0-no/mi
48	FREQ-RUM-REST-D2-#
49	FREQ-RUM-REST-D2-MIN
50	FREQ-RUM-REST-D2-no/mi
51	FREQ-RUM-REST-D11-#
52	FREQ-RUM-REST-D11-MIN
53	FREQ-RUM-REST-D11-no/m
54	FREQ-RUM-RUM-D0-#
55	FREQ-RUM-RUM-D0-no/min
56	FREQ-RUM-RUM-D0-no/min
57	FREQ-RUM-RUM-D2-#
58	FREQ-RUM-RUM-D2-MIN
59	FREQ-RUM-RUM-D2-no/min
60	FREQ-RUM-RUM-D11-#
61	FREQ-RUM-RUM-D11-MIN
62	FREQ-RUM-RUM-D11-no/mi
63	FREQ-RUM-FEED-D0-#
64	FREQ-RUM-FEED-D0-MIN
65	FREQ-RUM-FEED-D0-no/mi
66	FREQ-RUM-FEED-D2-#
67	FREQ-RUM-FEED-D2-MIN
68	FREQ-RUM-FEED-D2-no/mi
69	FREQ-RUM-FEED-D11-#
70	FREQ-RUM-FEED-D11-MIN
71	FREQ-RUM-FEED-D11-no/m
72	AMP-RET-REST-D0 mmHg
73	AMP-RET-REST-D2 mmHg
74	AMP-RET-REST-D11 mmHg
75	AMP-RET-REST-AVERAGE
76	AMP-RET-RUM-D0 mmHg
77	AMP-RET-RUM-D2 mmHg
78	AMP-RET-RUM-D11 mmHg
79	AMP-RET-RUM-AVERAGE
80	AMP-RET-FEED-D0 mmHg
81	AMP-RET-FEED-D2 mmHg
82	AMP-RET-FEED-D11 mmHg
83	AMP-RET-FEED-AVERAGE
84	AMP-RUM-REST-D0 mmHg
85	AMP-RUM-REST-D2 mmHg
86	AMP-RUM-REST-D11 mmH
87	AMP-RUM-REST-AVERAGE
88	AMP-RUM-RUM-D0 mmHg
89	AMP-RUM-RUM-D2 mmHg
90	AMP-RUM-RUM-D11 mmHg
91	AMP-RUM-RUM-AVERAGE
92	AMP-RUM-FEED-D0 mmHg
93	AMP-RUM-FEED-D2 mmHg
94	AMP-RUM-FEED-D11 mmH
95	AMP-RUM-FEED-AVERAGE
96	DUR-RET-REST-D0 sec
97	DUR-RET-REST-D2 sec
98	DUR-RET-REST-D11 sec
99	DUR-RET-REST-AVERAGE
100	DUR-RET-RUM-D0 sec

Column	Entry
101	DUR-RET-RUM-D2 sec
102	DUR-RET-RUM-D11sec
103	DUR-RET-RUM-AVERAGE
104	DUR-RET-FEED-D0 sec
105	DUR-RET-FEED-D2 sec
106	DUR-RET-FEED-D11 sec
107	DUR-RET-FEED-AVERAGE
108	DUR-RUM-REST-D0 sec
109	DUR-RUM-REST-D2 sec
110	DUR-RUM-REST-D11 sec
111	DUR-RUM-REST-AVERAGE
112	DUR-RUM-RUM-D0 sec
113	DUR-RUM-RUM-D2 sec
114	DUR-RUM-RUM-D11sec
115	DUR-RUM-RUM-AVERAGE
116	DUR-RUM-FEED-D0 sec
117	DUR-RUM-FEED-D2 sec
118	DUR-RUM-FEED-D11 sec
119	DUR-RUM-FEED-AVERAGE
120	Time at rest D0 %total
121	Time at rum D0 %total
122	Time at feed D0 %total
123	Time at rest D2 %total
124	Time at rum D2 %total
125	Time at feed D2 %total
126	Time at rest D11 %total
127	Time at rum D11 %total
128	Time at feed D11 %total
129	Overall freq RUM0
130	Overall freq RUM2
131	Overall freq RUM11
132	Overall freq RET0
133	Overall freq RET2
134	Overall freq RET11
135	Overall amp RUM0
136	Overall amp RUM2
137	Overall amp RUM11
138	Overall amp RET0
139	Overall amp RET2
140	Overall amp RET11
141	Overall dur RUM0
142	Overall dur RUM2
143	Overall dur RUM11
144	Overall dur RET0
145	Overall dur RET2
146	Overall dur RET11
147	VFA - DAY 0 A:P RATIO
148	VFA - DAY 0 A mg/100m
149	VFA - DAY 0 P mg/100m
150	VFA - DAY 0 IB mg/100m

Column	Entry
151	VFA - DAY 0 B mg/100m
152	VFA - DAY 0 IV mg/100m
153	VFA - DAY 0 V mg/100m
154	VFA - DAY 0 C mg/100m
155	VFA - DAY 0 TOTA mg/100m
156	VFA - DAY 2 A:P RATIO
157	VFA - DAY 2 A mg/100m
158	VFA - DAY 2 P mg/100m
159	VFA - DAY 2 IB mg/100m
160	VFA - DAY 2 B mg/100m
161	VFA - DAY 2 IV mg/100m
162	VFA - DAY 2 V mg/100m
163	VFA - DAY 2 C mg/100m
164	VFA - DAY 2 TOTA mg/100m
165	VFA - DAY 14 A:P RATIO
166	VFA - DAY 14 A mg/100m
167	VFA - DAY 14 P mg/100m
168	VFA - DAY 14 IB mg/100m
169	VFA - DAY 14 B mg/100m
170	VFA - DAY 14 IV mg/100m
171	VFA - DAY 14 V mg/100m
172	VFA - DAY 14 C mg/100m
173	VFA - DAY 14 TOT mg/100m
174	DM Feed at 60C
175	DM Feed at 110C
176	DM Feed Complete
177	DM Duo at 60C
178	DM Duo at 110C
179	DM Duo Complete
180	DM Fec at 60C
181	DM Fec at 110C
182	DM Fec Complete
183	Ash As-is Feed
184	Ash As-is Duo
185	Ash As-is Fec
186	Ash DM-basis Feed
187	Ash DM-basis Duo
188	Ash DM-basis Fec
189	Composit Feed DM
190	Composit Feed Ash
191	Composit Feed OM
192	Composit Feed NDF
193	Composit Feed ADF
194	Composit Feed ADL
195	Composit Feed cellulose
196	Composit Feed protein
197	Composit Feed Energy
198	Composit Duo DM
199	Composit Duo Ash
200	Composit Duo OM

Column	Entry		
201	Composit	Duo	NDF
202	Composit	Duo	ADF
203	Composit	Duo	ADL
204	Composit	Duo	cellulose
205	Composit	Duo	ptn-tot
206	Composit	Duo	ptn-bac
207	Composit	Duo	ptn-plant
208	Composit	Duo	[Cr]
209	Composit	Fec	DM
210	Composit	Fec	Ash
211	Composit	Fec	OM
212	Composit	Fec	NDF
213	Composit	Fec	ADF
214	Composit	Fec	ADL
215	Composit	Fec	cellulose
216	Composit	Fec	ptn
217	Composit	Fec	[Cr]
218	DM-Comp	Feed	Ash
219	DM-Comp	Feed	OM
220	DM-Comp	Feed	NDF
221	DM-Comp	Feed	ADF
222	DM-Comp	Feed	ADL
223	DM-Comp	Feed	cellulose
224	DM-Comp	Feed	protein
225	DM-Comp	Feed	Energy
226	DM-Comp	Duo	Ash
227	DM-Comp	Duo	OM
228	DM-Comp	Duo	NDF
229	DM-Comp	Duo	ADF
230	DM-Comp	Duo	ADL
231	DM-Comp	Duo	cellulose
232	DM-Comp	Duo	ptn-tot
233	DM-Comp	Duo	[Cr]
234	DM-Comp	Duo	DMflow
235	DM-Comp	Fec	Ash
236	DM-Comp	Fec	OM
237	DM-Comp	Fec	NDF
238	DM-Comp	Fec	ADF
239	DM-Comp	Fec	ADL
240	DM-Comp	Fec	cellulose
241	DM-Comp	Fec	ptn-tot
242	DM-Comp	Fec	[Cr]
243	DM-Comp	Fec	DMflow
244	DAPA	mg/g smp	bacteria
245	DAPA	mg/g smp	duodenal
246	DAPA	mg/g smp	fecal
247	Protein	%	bacteria
248	DAPA/PT	ratio	bacteria
249	DAPA	mg/g DM	duodenl
250	DAPA	mg/g DM	fecal

Column	Entry		
251	DAPA	mgbac/g	bacteria
252	FLOW	intake	feed
253	FLOW	weigh-bk	
254	FLOW	intake-asis	
255	FLOW	feed DM	
256	FLOW	corr DM-in	
257	INTAKE	Comp	DM
258	INTAKE	Comp	Ash
259	INTAKE	Comp	OM
260	INTAKE	Comp	NDF
261	INTAKE	Comp	ADF
262	INTAKE	Comp	ADL
263	INTAKE	Comp	cellulose
264	INTAKE	Comp	Protein
265	INTAKE	Comp	Energy
266	FLOWS	Duo	DM
267	FLOWS	Duo	Ash
268	FLOWS	Duo	OM
269	FLOWS	Duo	NDF
270	FLOWS	Duo	ADF
271	FLOWS	Duo	ADL
272	FLOWS	Duo	cellulose
273	FLOWS	Duo	ptn-tot
274	FLOWS	Duo	ptn-bac
275	FLOWS	Duo	ptn-plant
276	FLOWS	Fec	DM
277	FLOWS	Fec	Ash
278	FLOWS	Fec	OM
279	FLOWS	Fec	NDF
280	FLOWS	Fec	ADF
281	FLOWS	Fec	ADL
282	FLOWS	Fec	cellulose
283	FLOWS	Fec	protein
284	Feces	obs	wet g/d
285	Feces	obs	DM g/d
286	Feces	obs	DM intake
287	Feces	obs	digestibilit
288	Feces	obs	Ash
289	Feces	obs	OM
290	Feces	obs	NDF
291	Feces	obs	ADF
292	Feces	obs-flow	ADL
293	Feces	obs-flow	cellulose
294	Feces	obs-flow	protein
295	Total dig	obs	DM
296	Total dig	obs	Ash
297	Total dig	obs	OM
298	Total dig	obs	NDF
299	Total dig	obs	ADF
300	Total dig	obs	ADL

Column	Entry		
301	Total dig	obs	cellulose
302	Total dig	obs	protein
303	DM dig	ADL basi:	duodenal
304	DM dig	ADL basi:	fecal
305	Rumen	rate	
306	Rumen	volume	
307	Duodenal	constant	
308	Duodenal	coefficient	
309	Rumen di	percent	DM
310	Rumen di	percent	Ash
311	Rumen di	percent	OM
312	Rumen di	percent	NDF
313	Rumen di	percent	ADF
314	Rumen di	percent	ADL
315	Rumen di	percent	cellulose
316	Rumen di	percent	ptn-total
317	Rumen di	percent	ptn-plant
318	Total dig	percent	DM
319	Total dig	percent	Ash
320	Total dig	percent	OM
321	Total dig	percent	NDF
322	Total dig	percent	ADF
323	Total dig	percent	ADL
324	Total dig	percent	cellulose
325	Total dig	percent	protein
326	Bacteria	duo-est	DM g/d
327	Bacteria	duo-est	App DM g/d
328	Bacteria	duo-est	True DM g/d
329	Bacteria	duo-est	eff B/A
330	Bacteria	duo-est	eff B/T
331	Bacteria	fec-est	DM g/d
332	Bacteria	fec-est	App DM g/d
333	Bacteria	fec-est	True DM g/d
334	Bacteria	fec-est	eff B/A
335	Bacteria	fec-est	eff B/T
336	Bacteria	fec-obs	DM g/d
337	Bacteria	fec-obs	App DM g/d
338	Bacteria	fec-obs	True DM g/d
339	Bacteria	fec-obs	eff B/A
340	Bacteria	fec-obs	eff B/T
341	Duodenal	Cr	PPM
342	Duodenal	pool	g
343	Calorimetry	mL/min	O2
344	Calorimetry	mL/min	CO2
345	Calorimetry	mL/min	CH4
346	Methane loss		Mcal/d
347	Energy prod'n		Mcal/d
348	Protozoa #		Day 0
349	Protozoa #		Day 2
350	Protozoa #		Day 14

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
P	A	D	mm HIND	(WOOL LENGTH MEASURI BACK	FORELE	AVERAG	%RBC DAY 0	(HEMATOCRIT MEASURE DAY 2	DAY 14	AVERAG	(RUMEN FLUID pH MEASUREME DAY 0	DAY 2	DAY 14	AVERAG
I	211	0	30	25	30	28	29	31	28	29	6.59	6.12	6.74	6.48
I	222	1	25	30	30	28	35	36	32	34	6.51	6.43	6.84	6.59
I	225	2	36	34	40	37	31	32	34	32	6.42	6.33	6.59	6.45
I	233	4	40	34	40	38	28	31	28	29	6.64	5.81	6.62	6.36
II	211	4	36	30	38	35	29	30	27	29	6.72	5.84	6.03	6.20
II	222	2	42	35	42	40	29	31	31	30	6.77	6.56	6.34	6.56
II	225	0	45	35	45	42	27	31	32	30	6.74	6.67	6.55	6.65
II	233	1	43	35	43	40	34	27	30	30	6.72	6.57	6.42	6.57
III	211	2	18	15	20	18	30	29	27	29	6.58	6.38	6.67	6.54
III	222	4	20	20	22	21	34	35	31	33	6.67	6.29	6.65	6.54
III	225	1	20	22	24	22	32	34	35	34	6.85	6.59	6.69	6.71
III	233	0	22	24	26	24	28	29	30	29	6.78	6.54	6.41	6.58
IV	211	1	23	18	26	22	28	29	30	29	6.99	6.58	6.47	6.68
IV	222	0	26	25	27	26	34	34	35	34	6.49	6.42	6.50	6.47
IV	225	4	24	26	30	27	34	35	35	35	6.82	5.95	6.48	6.42
IV	233	2	28	28	35	30	29	30	30	30	6.85	6.29	6.65	6.60
AVERAGES														
		0	30.8	27.3	32.0	30.0	29.5	31.3	31.3	30.7	6.65	6.44	6.55	6.55
		1	27.8	26.3	30.8	28.3	32.3	31.5	31.8	31.8	6.77	6.54	6.61	6.64
		2	31.0	28.0	34.3	31.1	29.8	30.5	30.5	30.3	6.66	6.39	6.56	6.54
		4	30.5	27.5	32.5	30.2	31.3	32.8	30.3	31.4	6.71	5.97	6.45	6.38
	211		27.3	22.0	28.5	25.9	29.0	29.8	28.0	28.9	6.72	6.23	6.48	6.48
	222		28.3	27.5	30.3	28.7	33.0	34.0	32.3	33.1	6.61	6.43	6.58	6.54
	225		31.3	29.3	34.8	31.8	31.0	33.0	34.0	32.7	6.71	6.39	6.58	6.56
	233		33.3	30.3	36.0	33.2	29.8	29.3	29.5	29.5	6.75	6.30	6.53	6.53
I			32.8	30.8	35.0	32.8	30.8	32.5	30.5	31.3	6.54	6.17	6.70	6.47
II			42.0	33.8	42.0	39.3	29.8	29.8	30.0	29.8	6.74	6.41	6.34	6.49
III			20.0	20.3	23.0	21.1	31.0	31.8	30.8	31.2	6.72	6.45	6.61	6.59
IV			25.3	24.3	29.5	26.3	31.3	32.0	32.5	31.9	6.79	6.31	6.53	6.54

16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
		RESTINC	RETICULAR FREQUENCY								RUNINATING			
		DAY 0			DAY 2			DAY 11			DAY0			DAY 2
ANIMAL WEIGHT	OXYGEN CONSUM.	NUM	MIN	RATE	NUM	MIN	RATE	NUM	MIN	RATE	NUM	MIN	RATE	NUM
55.5	375.6	221	163	1.36	145	115	1.26	148	126	1.17	64	47	1.36	96
55.0	353.9	128	123	1.04	162	179	0.91	185	199	0.93	112	104	1.08	79
59.0	238.3	123	114	1.08	112	112	1	62	65	0.95	192	167	1.15	90
55.5	258.6	108	114	0.95	189	198	0.95	94	115	0.82	130	131	0.99	122
62.0	402.7	152	116	1.31	176	128	1.38	115	91	1.26	190	141	1.35	173
61.0	357.4	129	122	1.06	161	152	1.06	178	197	0.91	100	91	1.1	115
61.0	311.2	93	96	0.97	90	89	1.01	86	102	0.84	102	100	1.02	121
61.0	434.3	180	194	0.93	111	114	0.97	73	86	0.83	97	71	1.37	115
61.0	411.7	63	57	1.11	59	53	1.11	204	147	1.39	203	149	1.36	217
62.0	397.8	124	125	0.99	181	171	1.06	172	151	1.14	140	138	1.01	111
60.5	286.8	76	66	1.15	99	92	1.08	139	152	0.91	171	168	1.02	146
63.0	363.6	133	138	0.96	123	135	0.91	145	150	0.97	153	129	1.19	137
60.0	504.3	163	138	1.18	120	99	1.21	238	176	1.35	203	145	1.4	201
58.5	485.6	174	146	1.19	128	102	1.25	190	194	0.98	124	109	1.14	156
59.5	428.9	130	130	1	185	141	1.31	197	292	1.02	128	125	1.02	76
62.5	488.7	157	176	0.89	184	164	1.12	86	96	0.9	126	101	1.25	123
59.5	384	155	136	1.12	122	110	1.11	142	143	0.99	111	96	1.18	128
59.1	395	137	130	1.08	123	121	1.04	159	154	1.01	146	122	1.22	135
60.9	374	118	117	1.04	129	120	1.07	133	126	1.04	155	127	1.22	136
59.8	372	129	121	1.06	183	160	1.18	145	162	1.06	147	134	1.09	121
59.6	424	150	119	1.24	125	99	1.24	176	135	1.29	165	121	1.37	172
59.1	399	139	129	1.07	156	151	1.07	181	185	0.99	119	111	1.08	115
60.0	316	108	102	1.05	122	109	1.10	121	153	0.93	148	140	1.05	108
60.5	386	145	156	0.93	152	153	0.99	100	112	0.88	127	108	1.20	124
56.3	307	145	129	1.11	152	151	1.03	122	126	0.97	125	112	1.15	97
61.3	376	139	132	1.07	135	121	1.11	113	120	0.96	122	101	1.21	131
61.6	365	99	97	1.05	116	113	1.04	165	150	1.10	167	146	1.15	153
60.1	477	156	148	1.07	154	127	1.22	178	190	1.06	145	120	1.20	139

E	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45
						FEEDING									RESTING
	MIN	RATE	DAY 11 NUM	MIN	RATE	DAY0 NUM	MIN	RATE	DAY 2 NUM	MIN	RATE	DAY 11 NUM	MIN	RATE	DAY 0 NUM
	79	1.22	179	144	1.24	77	38	2.03	136	79	1.72	104	52	2	262
	82	0.96	82	68	1.21	79	46	1.72	89	66	1.34	108	85	1.27	124
	85	1.06	100	96	1.04	69	39	1.77	53	44	1.2	79	63	1.25	177
	102	1.2	82	73	1.12	57	34	1.68	65	49	1.33	17	12	1.42	150
	125	1.38	223	175	1.27	83	53	1.57	115	84	1.37	142	81	1.75	277
	104	1.11	92	92	1	89	55	1.62	79	52	1.52	66	48	1.42	216
	111	1.09	110	99	1.11	106	78	1.36	115	76	1.51	115	85	1.35	139
	94	1.22	140	119	1.18	74	42	1.76	70	43	1.63	75	42	1.79	230
	154	1.41	224	171	1.31	214	123	1.74	194	101	1.92	80	39	2.05	97
	99	1.12	132	113	1.17	73	49	1.49	92	58	1.59	85	52	1.63	193
	127	1.15	164	144	1.14	114	72	1.58	156	107	1.46	112	77	1.45	106
	121	1.13	148	124	1.19	76	44	1.73	95	60	1.58	76	44	1.73	192
	133	1.51	239	159	1.5	74	37	2	81	43	1.88	135	67	2.01	256
	139	1.12	194	158	1.23	76	41	1.85	120	68	1.76	76	43	1.77	290
	65	1.17	179	149	1.2	131	89	1.47	122	82	1.49	73	51	1.43	191
	100	1.23	230	180	1.28	74	40	1.85	116	65	1.78	67	39	1.72	158
	113	1.14	158	131	1.19	84	50	1.74	117	71	1.64	93	56	1.71	221
	109	1.21	156	123	1.26	85	49	1.77	99	65	1.58	108	68	1.63	179
	111	1.20	162	135	1.16	112	64	1.75	111	66	1.61	74	47	1.61	162
	96	1.22	154	128	1.19	86	56	1.55	99	68	1.45	79	49	1.56	203
	123	1.38	216	162	1.33	112	63	1.84	132	77	1.72	115	60	1.95	223
	106	1.08	125	108	1.15	79	48	1.67	95	61	1.55	84	57	1.52	206
	97	1.12	138	122	1.12	105	70	1.55	112	77	1.42	95	69	1.37	153
	104	1.20	150	124	1.19	70	40	1.76	87	54	1.58	59	34	1.67	183
	87	1.11	111	95	1.15	71	39	1.80	86	60	1.40	77	53	1.49	178
	109	1.20	141	121	1.14	88	57	1.58	95	64	1.51	100	64	1.58	216
	125	1.20	167	136	1.20	119	72	1.64	134	82	1.64	88	53	1.72	147
	109	1.26	211	162	1.30	89	52	1.79	110	65	1.73	88	50	1.73	224

	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
	RUMEN FREQUENCY								RUNINATING						
	MIN	RATE	DAY 2 NUM	MIN	RATE	DAY 11 NUM	MIN	RATE	DAY0 NUM	MIN	RATE	DAY 2 NUM	MIN	RATE	DAY 11 NUM
	125	2.1	249	115	2.17	171	105	1.63	102	47	2.17	163	79	2.06	216
	75	1.65	312	178	1.75	253	186	1.36	152	84	1.81	100	65	1.54	65
	114	1.55	132	77	1.71	98	65	1.51	243	143	1.7	142	85	1.67	142
	95	1.58	123	101	1.22	135	115	1.17	199	134	1.49	157	102	1.54	100
	116	2.39	250	128	1.95	161	95	1.69	300	141	2.13	253	125	2.02	266
	134	1.61	290	177	1.64	273	196	1.39	145	89	1.63	120	79	1.52	130
	96	1.45	115	89	1.29	97	91	1.07	126	100	1.26	150	111	1.35	133
	163	1.41	156	114	1.37	109	86	1.24	118	71	1.66	136	94	1.45	162
	57	1.7	87	48	1.81	248	147	1.9	292	149	1.96	215	117	1.84	294
	125	1.54	208	128	1.63	265	151	1.75	216	138	1.57	162	95	1.71	184
	66	1.61	144	92	1.57	170	152	1.12	292	168	1.74	206	127	1.62	206
	138	1.39	169	125	1.35	191	150	1.27	210	129	1.63	176	121	1.45	201
	138	1.86	177	98	1.81	290	153	1.9	238	129	1.84	176	78	2.26	249
	146	1.99	217	102	2.13	256	154	1.66	191	109	1.75	225	133	1.69	154
	130	1.47	165	98	1.68	312	214	1.46	197	125	1.58	98	65	1.51	165
	125	1.26	273	156	1.75	153	123	1.24	150	101	1.49	177	100	1.77	264
	126	1.73	188	108	1.74	179	125	1.41	157	96	1.70	179	111	1.64	176
	111	1.63	197	121	1.63	206	145	1.41	200	113	1.76	155	91	1.72	176
	108	1.53	196	115	1.73	193	133	1.51	208	121	1.70	164	95	1.70	208
	117	1.75	187	114	1.62	218	144	1.52	228	135	1.69	168	97	1.70	184
	109	2.01	191	97	1.94	218	125	1.78	233	117	2.03	202	100	2.05	261
	120	1.70	257	146	1.79	262	172	1.54	176	105	1.69	152	93	1.62	133
	102	1.52	139	89	1.56	169	131	1.29	215	134	1.57	149	97	1.54	162
	130	1.41	180	124	1.42	147	119	1.23	169	109	1.57	162	104	1.55	187
	102	1.72	204	118	1.71	164	118	1.42	174	102	1.79	141	83	1.70	131
	127	1.72	203	127	1.56	160	118	1.35	172	100	1.67	166	102	1.59	183
	97	1.56	152	96	1.59	219	150	1.51	253	146	1.73	190	115	1.66	221
	135	1.65	208	114	1.84	253	161	1.57	194	116	1.67	169	94	1.81	208

61	62	63	64	65	66	67	68	69	70	71	72	73	74	75
		FEEDING DAY0			DAY 2			DAY 11			AMPLITUDE RETICULUM RESTING			
MIN	RATE	NUM	MIN	RATE	NUM	MIN	RATE	NUM	MIN	RATE	DAY 0	DAY 2	DAY 11	AVG
134	1.61	117	36	3.08	208	79	2.63	131	52	2.52	25.4	14.1	38.6	26.0
39	1.67	91	41	2.22	156	66	2.36	157	77	2.04	41.3	69.0	96.5	68.9
96	1.48	61	21	2.9	83	44	1.89	145	71	2.04	29.5	8.8	18.1	18.8
62	1.61	76	34	2.24	100	49	2.04	40	21	1.9	39.0	36.8	68.5	48.4
171	1.67	121	41	2.95	193	74	2.61	217	81	2.68	14.4	29.8	16.8	20.3
92	1.41	144	52	2.77	113	52	2.17	111	46	2.31	71.9	49.6	32.7	51.4
79	1.68	180	78	2.31	159	66	2.41	185	85	2.14	37.5	37.3	33.0	35.9
119	1.53	104	42	2.48	129	43	3	122	42	2.9	70.3	43.7	21.4	45.1
171	1.72	313	123	2.54	262	101	2.59	117	39	3	92.0	30.1	43.6	55.2
114	1.61	121	49	2.47	118	46	2.57	143	52	2.75	71.7	72.0	33.5	59.1
144	1.43	210	72	2.92	264	107	2.47	194	77	2.52	76.7	27.4	84.3	62.8
124	1.62	127	44	2.89	149	60	2.48	118	44	2.68	37.0	83.6	53.5	58.0
115	2.17	130	37	3.51	126	43	2.96	190	67	2.93	67.4	57.0	43.0	55.8
81	1.9	128	41	3.12	208	68	3.06	104	34	3.06	53.8	54.9	71.9	60.2
115	1.43	220	89	2.47	116	54	2.15	110	51	2.16	63.6	71.7	91.2	75.5
180	1.47	94	33	2.85	188	65	2.89	110	39	2.82	66.4	60.0	41.9	56.1
105	1.70	138	50	2.85	181	68	2.65	135	54	2.60	38.4	47.5	49.3	45.1
104	1.70	134	48	2.78	169	65	2.70	167	66	2.60	63.9	49.3	61.3	58.2
135	1.52	153	57	2.77	162	66	2.39	121	49	2.54	65.0	37.1	34.1	45.4
116	1.58	135	53	2.53	132	56	2.34	128	51	2.37	46.9	53.1	52.5	50.8
148	1.79	170	60	3.02	198	74	2.70	165	60	2.78	49.8	32.8	35.5	39.4
82	1.65	121	46	2.65	149	58	2.54	129	53	2.54	59.7	61.4	58.7	59.9
109	1.51	168	65	2.65	156	68	2.23	159	71	2.22	51.8	36.3	56.7	48.3
121	1.56	100	36	2.62	142	54	2.60	98	37	2.58	52.9	56.5	46.3	51.9
83	1.59	86	34	2.61	137	60	2.23	118	55	2.13	33.6	32.7	55.4	40.6
115	1.57	137	53	2.63	149	59	2.55	159	64	2.51	48.5	40.1	26.0	38.2
138	1.60	193	72	2.71	196	79	2.53	143	53	2.74	69.4	53.3	53.7	58.8
123	1.74	143	50	2.99	160	58	2.77	130	48	2.74	62.8	60.9	62.0	61.9

76	77	78	79	80	81	82	83	84	85	86	87	88	89	90
								AMPLITUDE RUMEN RESTING						
RUMINATING				FEEDING							RUMINATING			
DAY 0	DAY 2	DAY 11	AVG	DAY 0	DAY 2	DAY 11	AVG	DAY 0	DAY 2	DAY 11	AVG	DAY 0	DAY 2	DAY 11
31.1	19.1	44.5	31.6	24.7	11.2	38.8	24.9	12.4	13.2	15.7	13.8	17.5	18.1	42.8
36.6	60.0	42.2	46.3	56.7	47.7	94.7	66.4	15.4	12.7	44.8	24.3	13	10.8	28.1
19.7	15.2	21.6	18.8	25.9	12.8	28.5	22.4	26	14.7	24.6	21.8	20	23.6	14.9
41.0	47.3	45.1	44.5	43.5	26.0	90.4	53.3	10.2	9.6	27.1	15.6	22	12.3	24.9
13.5	22.2	16.6	17.4	12.9	21.5	24.5	19.6	23.3	12.9	26.9	21.0	16.5	9.8	27.6
75.6	26.5	33.3	45.1	63.7	30.6	28.7	41.0	9.7	17.9	26.3	18.0	12.8	22.4	21.7
29.9	36.8	23.9	30.2	35.8	46.7	29.8	37.4	26.5	13.3	18.9	19.6	27.6	19.6	16.9
56.0	49.7	5.8	37.2	66.4	49.0	15.1	43.5	25	23.4	20.6	23.0	8.7	32.5	22.4
84.9	31.2	42.3	52.8	79.5	22.4	42.1	48.0	69.2	24.3	38.4	44.0	44.7	29.4	31.6
86.8	48.2	40.9	58.6	97.3	49.0	40.5	62.3	17.36	40.9	57.1	38.5	15.1	43.3	45.1
55.9	12.2	80.1	49.4	70.6	26.4	65.6	54.2	30.1	27.4	21	26.2	24.8	24.6	17.9
31.8	83.6	75.5	63.6	26.3	74.0	49.1	49.8	21.6	19.5	18.8	20.0	15	16.8	13.9
37.8	52.1	34.4	41.4	55.1	53.9	39.6	49.5	33.9	12.9	9.3	18.7	19.8	16.2	11.4
44.8	57.1	57.9	53.3	55.2	62.8	66.6	61.5	24	46.1	14.7	28.3	16.1	48.1	12.5
58.0	67.6	90.0	71.9	55.6	63.1	68.1	62.3	14.3	20.9	10.7	15.3	19.3	12.8	10.7
35.9	64.8	52.4	51.0	36.3	66.3	46.5	49.7	20.5	17.7	17.4	18.5	18.6	17.1	10.1
34.4	49.2	50.5	44.7	35.5	48.7	46.1	43.42	21.13	23.03	17.03	20.39	19.05	25.65	21.53
46.6	43.5	40.6	43.6	62.2	44.3	53.8	53.40	26.10	19.10	23.93	23.04	16.58	21.03	19.95
54.0	34.4	37.4	42.0	51.4	33.0	36.5	40.28	31.35	18.65	26.68	25.56	24.03	23.13	19.58
49.8	46.3	48.2	48.1	52.3	39.9	55.9	49.37	16.29	21.08	30.45	22.61	18.23	19.55	27.08
41.8	31.2	34.5	35.8	43.1	27.3	36.3	35.52	34.70	15.83	22.58	24.37	24.63	18.38	28.35
61.0	48.0	43.6	50.8	68.2	47.5	57.6	57.79	16.62	29.40	35.73	27.25	14.25	31.15	26.85
40.9	33.0	53.9	42.6	47.0	37.3	48.0	44.08	24.23	19.08	18.80	20.70	22.93	20.15	15.10
41.2	61.4	44.7	49.1	43.1	53.8	50.3	49.08	19.33	17.55	20.98	19.28	16.08	19.68	17.83
32.1	35.4	36.4	35.3	37.7	24.4	63.1	41.74	16.00	12.55	26.05	18.87	18.13	16.20	27.68
43.8	33.8	19.9	32.5	44.7	37.0	24.5	35.39	21.13	16.88	23.18	20.39	16.40	21.08	22.15
64.9	43.8	59.7	56.1	68.4	43.0	49.3	53.57	34.57	26.03	33.83	32.14	24.90	28.53	27.13
44.1	60.4	58.7	54.4	50.6	61.5	55.2	55.76	23.18	24.40	13.03	20.20	18.45	23.55	11.18

91	92	93	94	95	96	97	98	99	100	101	102	103	104	105
	FEEDING				DURATION RETICULUM RESTING				RUMINATING				FEEDING	
AVG	DAY 0	DAY 2	DAY 11	AVG	DAY 0	DAY 2	DAY 11	AVG	DAY 0	DAY 2	DAY 11	AVG	DAY 0	DAY 2
26.1	12	11.8	18.2	14.0	4.5	4.4	4.2	4.4	4.3	3.8	3.6	3.9	4.3	4.1
17.3	22.3	10.2	26	19.5	4.4	4.0	3.8	4.1	4.3	4.1	3.8	4.1	4.7	4.4
19.5	23.6	15.7	17	18.8	5.5	4.0	4.4	4.6	4.7	4.0	3.8	4.2	6.3	4.0
19.7	8.7	11.4	26.9	15.7	6.5	3.9	4.1	4.8	4.9	3.9	3.3	4.0	5.6	4.4
18.0	17.2	15.6	25.5	19.4	4.0	4.2	4.1	4.1	3.7	3.5	3.7	3.6	4.2	4.0
19.0	32.6	24.8	43	33.5	3.9	3.9	4.3	4.0	3.7	3.9	3.9	3.8	4.2	4.1
21.4	24.6	24.8	23.8	24.4	4.6	4.8	4.6	4.7	3.9	4.0	3.8	3.9	4.8	4.7
21.2	20.2	32.2	18.4	23.6	4.2	4.5	4.2	4.3	4.2	3.8	4.1	4.0	4.1	3.7
35.2	22.9	23.5	37.2	27.9	4.5	4.3	4.0	4.3	4.0	4.3	3.8	4.0	4.4	3.9
34.5	22.7	52.6	56.1	43.8	4.0	4.9	4.3	4.4	3.9	4.9	4.1	4.3	4.2	4.2
22.4	9.3	28.6	43.7	27.2	4.3	4.1	4.5	4.3	3.7	4.1	4.0	3.9	4.1	4.0
15.2	18.4	23.2	8.9	16.8	4.2	4.1	4.6	4.3	4.1	4.1	4.0	4.1	4.1	4.0
15.8	26.3	18.6	17.1	20.7	4.5	4.2	4.1	4.3	3.8	4.2	3.8	3.9	4.2	4.2
25.6	39.1	51.3	11	33.8	4.2	4.6	4.1	4.3	4.0	4.6	3.7	4.1	4.3	4.2
14.3	19.3	18.6	16.7	18.2	4.3	4.2	4.3	4.3	4.0	4.2	4.2	4.1	4.3	4.7
15.3	20.5	17.5	11.4	16.5	4.3	4.5	4.3	4.4	3.9	4.5	3.8	4.1	3.9	4.3
22.08	23.53	27.78	15.48	22.26	4.38	4.48	4.38	4.41	4.08	4.13	3.78	3.99	4.38	4.25
19.18	19.53	22.40	26.30	22.74	4.35	4.20	4.15	4.23	4.00	4.05	3.93	3.99	4.28	4.08
22.24	24.90	20.38	27.15	24.14	4.55	4.18	4.25	4.33	4.08	4.18	3.83	4.03	4.70	4.08
21.62	16.98	24.55	31.30	24.28	4.70	4.30	4.20	4.40	4.13	4.13	3.83	4.03	4.58	4.33
23.78	19.60	17.38	24.50	20.49	4.38	4.28	4.10	4.25	3.95	3.95	3.73	3.88	4.28	4.05
24.08	29.18	34.73	34.03	32.64	4.13	4.35	4.13	4.20	3.98	4.38	3.88	4.08	4.35	4.23
19.39	19.20	21.93	25.30	22.14	4.68	4.28	4.45	4.47	4.08	4.08	3.95	4.03	4.88	4.35
17.86	16.95	21.08	16.40	18.14	4.80	4.25	4.30	4.45	4.28	4.08	3.80	4.05	4.43	4.10
20.67	16.65	12.28	22.03	16.98	5.23	4.08	4.13	4.48	4.55	3.95	3.63	4.04	5.23	4.23
19.88	23.65	24.35	27.68	25.23	4.18	4.35	4.30	4.28	3.88	3.80	3.88	3.85	4.33	4.13
26.85	18.33	31.98	36.48	28.93	4.25	4.35	4.35	4.32	3.93	4.35	3.98	4.08	4.20	4.03
17.73	26.30	26.50	14.05	22.28	4.33	4.38	4.20	4.30	3.93	4.38	3.88	4.06	4.18	4.35

106	107	108	109	110	111	112	113	114	115	116	117	118	119	120
		DURATION RUMEN RESTING			RUMINATING				FEEDING					Time spe Day 0 Resting
DAY 11	AVG	DAY 0	DAY 2	DAY 11	AVG	DAY 0	DAY 2	DAY 11	AVG	DAY 0	DAY 2	DAY 11	AVG	
4.1	4.2	5.0	5.3	5.2	5.2	5.1	4.7	4.8	4.9	5.1	5.1	3.7	4.6	0.657
4.1	4.4	5.3	5.5	3.8	4.9	5.9	5.5	5.2	5.5	5.7	4.9	5.0	5.2	0.451
4.1	4.8	6.6	5.5	5.5	5.9	6.1	5.5	5.0	5.5	7.3	5.5	5.5	6.1	0.356
4.3	4.8	5.0	5.5	5.3	5.3	4.8	5.5	5.3	5.2	5.6	5.4	5.6	5.5	0.409
3.9	4.0	4.6	5.1	6.2	5.3	4.7	5.1	6.2	5.3	4.8	4.7	5.4	5.0	0.374
4.1	4.1	4.7	3.7	4.8	4.4	4.9	3.7	4.8	4.5	5.5	5.2	5.5	5.4	0.455
4.6	4.7	5.5	5.0	5.3	5.3	6.0	5.0	5.3	5.4	6.0	5.9	5.8	5.9	0.350
4.4	4.1	5.6	4.9	5.5	5.3	5.7	5.3	5.9	5.6	5.4	4.8	5.5	5.2	0.632
4.2	4.2	5.7	5.3	5.2	5.4	5.7	4.5	5.3	5.2	5.7	4.8	4.1	4.9	0.173
4.2	4.2	5.5	4.9	5.0	5.1	5.4	5.3	4.7	5.1	5.5	5.6	5.5	5.5	0.401
4.4	4.2	6.1	5.1	5.2	5.5	6.3	5.2	6.2	5.9	5.8	5.6	6.2	5.9	0.216
4.4	4.2	5.2	4.9	4.7	4.9	5.2	5.9	5.1	5.4	5.2	6.1	6.6	6.0	0.444
4.1	4.2	5.8	5.1	7.7	6.2	5.0	4.4	5.2	4.9	4.7	5.1	6.0	5.3	0.431
4.1	4.2	5.8	5.5	5.2	5.5	4.6	6.3	4.8	5.2	5.8	5.8	5.7	5.8	0.493
4.0	4.3	5.7	5.3	4.6	5.2	5.1	4.9	5.3	5.1	5.8	5.0	5.5	5.4	0.378
4.2	4.1	5.2	5.9	4.9	5.3	5.2	4.9	5.1	5.1	5.1	5.8	5.4	5.4	0.555
4.30	4.31	5.38	5.18	5.10	5.22	5.23	5.48	5.00	5.23	5.53	5.73	5.45	5.57	0.486
4.25	4.20	5.70	5.15	5.55	5.47	5.73	5.10	5.63	5.48	5.40	5.10	5.68	5.39	0.432
4.15	4.31	5.55	5.10	5.10	5.25	5.48	4.65	5.05	5.06	5.90	5.33	5.13	5.45	0.385
4.10	4.33	5.20	5.20	5.28	5.23	5.00	5.20	5.38	5.19	5.43	5.18	5.50	5.37	0.390
4.08	4.13	5.28	5.20	6.08	5.52	5.13	4.68	5.38	5.06	5.08	4.93	4.80	4.93	0.409
4.13	4.23	5.33	4.90	4.70	4.98	5.20	5.20	4.88	5.09	5.63	5.38	5.43	5.48	0.450
4.28	4.50	5.98	5.23	5.15	5.45	5.88	5.15	5.45	5.49	6.23	5.50	5.75	5.83	0.325
4.33	4.28	5.25	5.30	5.10	5.22	5.23	5.40	5.35	5.33	5.33	5.53	5.78	5.54	0.510
4.15	4.53	5.48	5.45	4.95	5.29	5.48	5.30	5.08	5.28	5.93	5.23	4.95	5.37	0.468
4.25	4.23	5.10	4.68	5.45	5.08	5.33	4.78	5.55	5.22	5.43	5.15	5.55	5.38	0.453
4.30	4.18	5.63	5.05	5.03	5.23	5.65	5.23	5.33	5.40	5.55	5.53	5.60	5.56	0.308
4.10	4.21	5.63	5.45	5.60	5.56	4.98	5.13	5.10	5.07	5.35	5.43	5.65	5.48	0.464

121	122	123	124	125	126	127	128	129	130	131	132	133	134	135
								FREQUENCIES						AMPLITU
rumnatin	feeding	Day 2 Resting	rumnatin	feeding	Day 11 Resting	rumnatin	feeding	RUM0	RUM2	RUM11	RET0	RET2	RET11	RUM0
0.190	0.153	0.421	0.289	0.289	0.391	0.447	0.161	2.26	2.27	1.76	1.46	1.38	1.34	13.3
0.381	0.168	0.547	0.251	0.202	0.565	0.193	0.241	1.81	1.82	1.58	1.17	1.01	1.07	15.6
0.522	0.122	0.465	0.353	0.183	0.290	0.429	0.281	1.79	1.73	1.65	1.20	1.06	1.07	22.6
0.470	0.122	0.567	0.292	0.140	0.575	0.365	0.060	1.62	1.43	1.37	1.06	1.08	0.97	15.6
0.455	0.171	0.380	0.371	0.249	0.262	0.504	0.233	2.37	2.14	1.91	1.37	1.38	1.38	19.2
0.340	0.205	0.494	0.338	0.169	0.585	0.273	0.142	1.85	1.69	1.53	1.19	1.15	1.01	15.5
0.365	0.285	0.322	0.402	0.275	0.357	0.346	0.297	1.63	1.62	1.60	1.10	1.18	1.09	26.4
0.231	0.137	0.454	0.375	0.171	0.353	0.478	0.169	1.61	1.68	1.66	1.15	1.18	1.16	20.6
0.453	0.374	0.172	0.500	0.328	0.412	0.479	0.109	2.13	2.08	1.93	1.46	1.53	1.42	40.8
0.442	0.157	0.521	0.302	0.177	0.478	0.358	0.165	1.70	1.82	1.86	1.08	1.17	1.23	17.2
0.549	0.235	0.282	0.390	0.328	0.408	0.386	0.206	1.99	1.88	1.53	1.18	1.23	1.11	22.3
0.415	0.141	0.427	0.383	0.190	0.472	0.390	0.138	1.70	1.60	1.60	1.16	1.12	1.16	18.4
0.453	0.116	0.360	0.484	0.156	0.438	0.396	0.167	2.04	2.21	2.18	1.37	1.46	1.52	26.6
0.368	0.139	0.330	0.450	0.220	0.491	0.400	0.109	2.06	2.14	1.91	1.26	1.30	1.17	23.2
0.363	0.259	0.490	0.226	0.285	0.593	0.303	0.104	1.77	1.76	1.52	1.13	1.33	1.12	17.4
0.319	0.126	0.498	0.304	0.198	0.305	0.571	0.124	1.53	1.98	1.57	1.13	1.28	1.22	19.9
0.334	0.179	0.375	0.381	0.244	0.428	0.396	0.176	1.912	1.908	1.718	1.247	1.247	1.187	20.3
0.404	0.164	0.411	0.375	0.214	0.441	0.363	0.196	1.863	1.899	1.737	1.217	1.219	1.214	21.3
0.408	0.207	0.407	0.374	0.219	0.398	0.438	0.164	1.828	1.870	1.668	1.243	1.255	1.181	24.7
0.433	0.177	0.490	0.298	0.213	0.477	0.382	0.140	1.863	1.791	1.668	1.159	1.239	1.173	17.3
0.388	0.203	0.333	0.411	0.256	0.376	0.457	0.168	2.201	2.176	1.947	1.417	1.436	1.414	25.0
0.383	0.167	0.473	0.335	0.192	0.530	0.306	0.164	1.855	1.867	1.721	1.175	1.160	1.118	17.9
0.450	0.225	0.390	0.343	0.268	0.412	0.366	0.222	1.794	1.753	1.574	1.152	1.200	1.096	22.2
0.359	0.132	0.487	0.338	0.175	0.426	0.451	0.123	1.617	1.673	1.550	1.123	1.165	1.126	18.6
0.390	0.141	0.500	0.296	0.204	0.455	0.358	0.186	1.870	1.812	1.592	1.223	1.131	1.110	16.8
0.348	0.199	0.412	0.371	0.216	0.389	0.400	0.210	1.866	1.783	1.674	1.201	1.222	1.158	20.4
0.465	0.227	0.351	0.394	0.256	0.442	0.403	0.155	1.881	1.847	1.732	1.220	1.263	1.232	24.7
0.376	0.160	0.420	0.366	0.215	0.457	0.417	0.126	1.851	2.026	1.794	1.223	1.344	1.255	21.8

136	137	138	139	140	141	142	143	144	145	146	147	148	149	150
nt on each activity (as % total)					DURATIONS							VOLATILE FATTY ACID AN		
RUM2	RUM11	RET0	RET2	RET11	RUM0	RUM2	RUM11	RET0	RET2	RET11	A:P	DAY 0 A	DAY 0 P	DAY 0 IB
14.2	28.2	26.4	14.7	41.3	5.03	5.07	4.78	4.43	4.14	3.92	3.36	378.17	112.58	3.75
11.7	37.0	42.1	62.4	85.6	5.60	5.38	4.36	4.41	4.11	3.87	3.34	347.56	103.94	5.11
18.0	18.3	23.9	11.8	22.5	6.42	5.50	5.29	5.18	4.00	4.06	3.00	329.79	110.10	4.79
10.6	26.3	40.1	39.5	61.3	4.98	5.49	5.32	5.64	3.97	3.82	3.08	294.31	95.46	5.26
12.4	26.9	13.7	24.9	18.5	4.68	5.00	6.01	3.90	3.89	3.85	2.72	256.07	94.13	3.97
20.6	27.4	71.5	38.6	32.3	4.93	3.95	4.90	3.89	3.93	4.16	2.89	304.55	105.21	5.90
19.0	19.7	34.2	39.7	28.9	5.82	5.25	5.45	4.40	4.45	4.32	0.00	.	.	.
28.3	21.1	66.5	46.9	12.9	5.60	5.03	5.69	4.19	4.10	4.19	0.00	.	.	.
26.6	35.0	84.1	28.1	42.8	5.70	4.74	5.13	4.24	4.17	3.93	3.15	324.52	103.17	5.58
43.7	52.6	82.4	60.7	37.3	5.46	5.14	4.98	3.99	4.78	4.21	3.26	301.48	92.52	6.15
26.7	24.5	63.8	21.2	78.8	6.14	5.30	5.79	3.92	4.07	4.29	3.19	262.42	82.16	5.38
19.2	15.5	33.3	61.8	61.5	5.20	5.51	5.12	4.14	4.08	4.34	3.28	281.39	85.76	5.67
15.4	11.4	52.6	54.1	39.0	5.31	4.76	6.43	4.15	4.20	3.98	3.37	286.16	84.89	5.58
48.1	13.4	50.7	57.6	65.7	5.36	5.93	5.09	4.14	4.51	3.94	3.59	310.11	86.44	4.66
18.4	11.3	59.5	68.3	88.4	5.51	5.12	4.91	4.19	4.34	4.24	2.84	280.83	98.88	5.82
17.5	12.5	52.9	62.7	48.5	5.19	5.58	5.08	4.12	4.46	4.00	3.37	272.58	80.85	5.81
25.1	19.2	36.2	48.5	49.3	5.35	5.44	5.11	4.28	4.30	4.13	2.557	242.42	71.20	3.521
20.5	23.5	56.2	46.1	54.1	5.66	5.12	5.57	4.17	4.12	4.08	2.477	224.03	67.75	4.017
20.7	23.3	58.1	35.3	36.5	5.56	4.94	5.10	4.36	4.14	4.04	3.102	307.86	99.83	5.520
21.3	29.3	48.9	48.4	51.4	5.16	5.19	5.30	4.43	4.24	4.03	2.976	283.17	95.25	5.300
17.2	25.4	44.2	30.5	35.4	5.18	4.89	5.59	4.18	4.10	3.92	3.149	311.23	98.69	4.718
31.0	32.6	61.7	54.9	55.2	5.34	5.10	4.83	4.11	4.33	4.05	3.271	315.92	97.03	5.456
20.5	18.4	45.4	35.2	54.7	5.97	5.29	5.36	4.42	4.22	4.23	2.257	218.26	72.79	3.999
18.9	18.8	48.2	57.7	46.0	5.24	5.40	5.30	4.52	4.15	4.09	2.434	212.07	65.52	4.186
13.6	27.5	33.1	32.1	52.7	5.51	5.36	4.94	4.92	4.05	3.92	3.195	337.46	105.52	4.726
20.1	23.8	46.5	37.5	23.1	5.26	4.81	5.51	4.09	4.09	4.13	1.404	140.15	49.83	2.468
29.0	31.9	65.9	48.0	55.1	5.62	5.17	5.25	4.07	4.27	4.19	3.220	292.45	90.90	5.696
24.9	12.2	53.9	60.7	60.4	5.34	5.35	5.38	4.15	4.38	4.04	3.292	287.42	87.77	5.470

151	152	153	154	155	156	157	158	159	160	161	162	163	164	165
IDES														
DAY 0 B	DAY 0 IV	DAY 0 V	DAY 0 C	DAY 0 TOTAL	A:P	DAY 2 A	DAY 2 P	DAY 2 IB	DAY 2 B	DAY 2 IV	DAY 2 V	DAY 2 C	DAY 2 TOTAL	A:P
45.72	2.84	6.61	1.03	550.70	3.15	358.01	113.68	2.38	60.53	1.81	7.35	0.45	544.21	2.68
55.83	5.22	6.96	1.13	525.75	2.82	315.33	112.00	3.92	62.64	3.68	8.25	1.07	506.90	2.52
47.24	4.92	6.73	0.75	504.33	2.50	268.97	107.40	1.61	45.82	1.48	6.86	0.76	432.90	2.19
45.13	6.69	6.33	1.43	454.62	2.72	431.26	158.45	2.40	60.49	3.32	9.35	1.35	666.62	2.58
35.28	3.85	8.02	0.46	401.78	3.13	438.71	139.99	0.59	82.52	1.66	11.40	2.12	677.00	2.80
39.57	6.44	7.40	0.72	469.78	2.66	255.62	95.96	2.31	47.56	2.00	6.31	1.11	410.86	2.73
.	.	.	.	.	2.40	292.31	121.72	4.67	47.77	5.44	8.72	0.96	481.59	2.71
.	.	.	.	.	2.84	339.69	119.41	5.30	54.49	6.60	8.66	1.48	535.64	2.84
47.87	7.36	7.05	0.81	496.36	2.53	293.08	115.94	2.80	55.96	3.60	9.10	0.58	481.05	2.48
42.50	7.67	4.85	1.25	456.42	2.65	350.90	132.32	1.89	66.57	1.39	10.84	1.54	565.45	3.42
39.39	5.77	4.85	0.62	400.59	2.39	245.01	102.62	2.52	50.21	2.57	5.05	0.57	408.55	2.23
45.20	6.65	5.99	1.30	431.97	2.90	268.59	92.67	3.70	57.00	4.49	5.45	1.62	433.52	2.87
41.36	6.21	4.67	0.62	429.48	2.83	310.92	109.94	4.41	56.56	4.85	6.26	0.79	493.73	2.66
39.13	6.07	5.14	1.04	452.60	3.22	350.37	108.91	3.18	59.86	3.37	5.85	1.56	533.10	3.16
42.39	6.73	6.17	0.59	441.42	2.78	415.78	149.67	1.91	62.71	2.01	10.66	0.81	643.54	3.01
41.67	6.83	5.05	1.49	414.29	2.92	321.31	110.02	2.60	51.50	2.82	6.45	1.21	496.10	3.41
32.512	3.892	4.436	0.842	358.8	2.917	317.32	109.25	3.48	56.291	3.777	6.842	1.150	498.11	2.86
34.145	4.298	4.121	0.591	339.0	2.719	302.74	110.99	4.04	55.976	4.426	7.057	0.980	486.20	2.56
44.087	6.387	6.557	0.944	471.2	2.654	284.75	107.33	2.38	50.206	2.475	7.179	0.915	455.23	2.70
41.327	6.236	6.343	0.934	438.6	2.821	409.16	145.11	1.70	68.071	2.095	10.564	1.454	638.15	2.95
42.558	5.064	6.587	0.729	469.6	2.910	350.18	119.89	2.54	63.891	2.982	8.528	0.987	549.00	2.65
44.256	6.348	6.089	1.038	476.1	2.837	318.05	112.30	2.83	59.156	2.611	7.812	1.321	504.08	2.96
32.256	4.355	4.436	0.490	336.6	2.518	305.52	120.35	2.68	51.626	2.873	7.823	0.777	491.65	2.54
33.001	5.045	4.344	1.055	325.2	2.846	340.21	120.14	3.55	55.872	4.306	7.478	1.413	532.97	2.93
48.483	4.917	6.657	1.088	508.8	2.798	343.39	122.88	2.57	57.370	2.574	7.952	0.908	537.66	2.49
18.712	2.572	3.855	0.296	217.9	2.761	331.58	119.27	3.22	58.086	3.924	8.773	1.420	526.27	2.77
43.741	6.862	5.687	0.995	446.3	2.616	289.39	110.89	2.73	57.434	3.011	7.612	1.078	472.15	2.75
41.136	6.461	5.259	0.933	434.4	2.936	349.59	119.63	3.08	57.655	3.263	7.304	1.092	541.62	3.06

166	167	168	169	170	171	172	173	174	175	176	177	178	179	180
ALYSIS OF RUMEN FLUID (mg/100mL)								DRY MATTER VALUES						
DAY 14 A	DAY 14 P	DAY 14 IB	DAY 14 B	DAY 14 IV	DAY 14 V	DAY 14 C	DAY 14 TOTAL	FEED SAMPLES @60C	@100C	Complete	DUODENAL SAMPLES @60C	@100C	Complete	INITIAL
258.60	96.65	2.60	40.19	3.05	5.31	1.03	407.42	0.8877	0.9540	0.8468	0.0495	0.9747	0.0482	0.3614
251.06	99.58	3.88	36.75	4.90	4.33	0.00	400.50	0.8877	0.9540	0.8468	0.0568	0.9716	0.0552	0.4068
308.61	141.03	3.09	49.89	3.18	6.79	0.00	512.59	0.8877	0.9540	0.8468	0.0555	0.9764	0.0542	0.4008
280.48	108.52	3.00	48.62	3.82	6.06	0.92	451.43	0.8877	0.9540	0.8468	0.0466	0.9747	0.0454	0.3944
366.22	130.80	2.33	78.49	3.50	11.05	1.46	593.85	0.8935	0.9550	0.8533	0.0511	0.9812	0.0501	0.3903
259.57	94.97	3.09	42.31	3.72	6.04	0.49	410.19	0.8935	0.9550	0.8533	0.0543	0.9745	0.0529	0.3777
201.23	74.13	5.05	46.50	6.99	4.72	1.19	339.80	0.8935	0.9550	0.8533	0.0576	0.9768	0.0563	0.3739
334.14	117.47	3.88	57.92	4.40	8.66	1.56	528.04	0.8935	0.9550	0.8533	0.0433	0.9791	0.0424	0.4224
285.45	114.94	2.60	49.08	2.50	7.74	0.00	462.30	0.8899	0.9546	0.8495	0.0415	0.9839	0.0408	0.4020
287.75	84.17	2.19	50.89	2.26	7.51	1.17	435.95	0.8899	0.9546	0.8495	0.0508	0.9865	0.0501	0.3545
181.38	81.31	3.27	36.03	4.98	3.56	0.43	310.96	0.8899	0.9546	0.8495	0.0400	0.9802	0.0392	0.4825
281.70	98.01	3.71	52.64	5.46	6.24	0.82	448.59	0.8899	0.9546	0.8495	0.0357	0.9822	0.0351	0.4065
329.80	124.08	3.57	59.79	3.79	9.07	0.53	530.62	0.8759	0.9533	0.8360	0.0380	0.9635	0.0366	0.4304
311.23	98.36	4.88	53.40	6.51	6.41	0.63	481.42	0.8759	0.9533	0.8350	0.0487	0.9615	0.0468	0.3716
298.71	99.33	2.46	67.11	2.09	7.47	1.33	478.50	0.8759	0.9533	0.8350	0.0377	0.9669	0.0364	0.3600
213.35	62.54	0.00	42.03	2.52	6.25	0.00	326.69	0.8759	0.9533	0.8350	0.0337	0.9621	0.0324	0.4376
263.19	91.79	4.061	48.182	5.502	5.669	0.917	419.31	0.89	0.954	0.846	0.048	0.974	0.047	0.378
274.10	105.61	3.650	47.623	4.519	6.404	0.630	442.53	0.89	0.954	0.846	0.045	0.974	0.043	0.436
266.74	103.37	2.195	45.827	2.980	6.706	0.121	427.94	0.89	0.954	0.846	0.046	0.974	0.045	0.405
308.29	105.71	2.495	61.279	2.920	8.023	1.220	489.93	0.89	0.954	0.846	0.047	0.977	0.046	0.375
310.01	116.61	2.778	56.886	3.212	8.292	0.754	498.55	0.89	0.954	0.846	0.045	0.976	0.044	0.396
277.40	94.27	3.509	45.839	4.350	6.073	0.571	432.02	0.89	0.954	0.846	0.053	0.974	0.051	0.378
247.48	98.95	3.466	49.882	4.311	5.634	0.737	410.47	0.89	0.954	0.846	0.048	0.975	0.047	0.404
277.42	96.64	2.649	50.304	4.048	6.804	0.825	438.69	0.89	0.954	0.846	0.040	0.975	0.039	0.415
274.69	111.45	3.141	43.862	3.740	5.622	0.486	442.99	0.89	0.954	0.847	0.052	0.974	0.051	0.391
290.29	104.34	3.588	56.307	4.654	7.617	1.174	467.97	0.89	0.955	0.853	0.052	0.978	0.050	0.391
259.07	94.61	2.944	47.160	3.801	6.264	0.606	414.45	0.89	0.955	0.849	0.042	0.983	0.041	0.411
288.27	96.08	2.728	55.582	3.726	7.299	0.621	454.31	0.88	0.953	0.835	0.040	0.964	0.038	0.400

181	182	183	184	185	186	187	188	189	190	191	192	193	194	195
		ASH VALUES AS IS			ASH VALUES DM BASIS			FEED COMPOSITION - as is						
FECAL	SAMPLES	FEED	DUODEN	FECAL	FEED	DUODEN	FECAL	DM	ASH	OM	NDF	ADF	ADL	cellulose
FINAL	Complete													
								FDDM	FDASH	FDOM	FDNDF	FDADF	FDADL	FDCLL
0.9644	0.3485	8.89	17.29	12.84	9.317	17.742	13.315	0.8468	0.0889	0.9111	0.5504	0.3345	0.0510	0.2834
0.9628	0.3917	8.89	15.66	11.98	9.317	16.121	12.448	0.8468	0.0889	0.9111	0.5504	0.3345	0.0510	0.2834
0.9655	0.3869	8.89	14.17	11.38	9.317	14.509	11.782	0.8468	0.0889	0.9111	0.5504	0.3345	0.0510	0.2834
0.9658	0.3809	8.89	17.19	10.67	9.317	17.640	11.048	0.8468	0.0889	0.9111	0.5504	0.3345	0.0510	0.2834
0.9617	0.3753	8.81	16.42	11.89	9.222	16.737	12.365	0.8533	0.0881	0.9119	0.5493	0.3314	0.0504	0.2810
0.9496	0.3587	8.81	16.16	12.27	9.222	16.581	12.918	0.8533	0.0881	0.9119	0.5493	0.3314	0.0504	0.2810
0.9623	0.3598	8.81	15.21	13.58	9.222	15.570	14.107	0.8533	0.0881	0.9119	0.5493	0.3314	0.0504	0.2810
0.9585	0.4048	8.81	18.46	12.64	9.222	18.849	13.190	0.8533	0.0881	0.9119	0.5493	0.3314	0.0504	0.2810
0.9652	0.3880	9.52	20.89	12.62	9.974	21.235	13.079	0.8495	0.0952	0.9048	0.5372	0.3222	0.0508	0.2715
0.9682	0.3432	9.52	17.07	11.94	9.974	17.307	12.328	0.8495	0.0952	0.9048	0.5372	0.3222	0.0508	0.2715
0.9617	0.4640	9.52	21.53	11.39	9.974	21.969	11.847	0.8495	0.0952	0.9048	0.5372	0.3222	0.0508	0.2715
0.9628	0.3914	9.52	22.66	14.17	9.974	23.074	14.717	0.8495	0.0952	0.9048	0.5372	0.3222	0.0508	0.2715
0.9644	0.4151	9.40	22.87	12.20	9.865	23.732	12.655	0.8350	0.0940	0.9060	0.5162	0.3069	0.0477	0.2592
0.9618	0.3574	9.40	18.37	14.34	9.865	19.104	14.906	0.8350	0.0940	0.9060	0.5162	0.3069	0.0477	0.2592
0.9616	0.3462	9.40	20.69	11.12	9.865	21.395	11.568	0.8350	0.0940	0.9060	0.5162	0.3069	0.0477	0.2592
0.9611	0.4206	9.40	23.49	11.22	9.865	24.419	11.674	0.8350	0.0940	0.9060	0.5162	0.3069	0.0477	0.2592
0.963	0.364	9.155	18.383	13.731	9.594	18.873	14.261	0.846	0.092	0.908	0.538	0.324	0.050	0.274
0.962	0.419	9.155	19.830	12.056	9.594	20.168	12.535	0.846	0.092	0.908	0.538	0.324	0.050	0.274
0.960	0.389	9.155	18.678	11.871	9.594	19.186	12.363	0.846	0.092	0.908	0.538	0.324	0.050	0.274
0.964	0.361	9.155	17.844	11.405	9.594	18.270	11.827	0.846	0.092	0.908	0.538	0.324	0.050	0.274
0.964	0.382	9.155	19.369	12.390	9.594	19.862	12.853	0.846	0.092	0.908	0.538	0.324	0.050	0.274
0.961	0.363	9.155	16.816	12.631	9.594	17.278	13.150	0.846	0.092	0.908	0.538	0.324	0.050	0.274
0.963	0.389	9.155	17.899	11.867	9.594	18.361	12.326	0.846	0.092	0.908	0.538	0.324	0.050	0.274
0.962	0.399	9.155	20.452	12.175	9.594	20.996	12.657	0.846	0.092	0.908	0.538	0.324	0.050	0.274
0.965	0.377	8.888	16.079	11.718	9.317	16.503	12.148	0.847	0.089	0.911	0.550	0.334	0.051	0.283
0.958	0.375	8.807	16.561	12.594	9.222	16.934	13.145	0.853	0.088	0.912	0.549	0.331	0.050	0.281
0.964	0.397	9.521	20.540	12.530	9.974	20.896	12.993	0.849	0.095	0.905	0.537	0.322	0.051	0.271
0.962	0.385	9.404	21.354	12.221	9.865	22.163	12.701	0.835	0.094	0.906	0.516	0.307	0.048	0.259

196	197	198	199	200	201	202	203	204	205	206	207	208	209	210
		DUODENAL SAMPLE COMPOSITION - as observed											FECAL SAMPLE C	
PROTEIN	Energy	DM	ASH	OM	NDF	ADF	ADL	cellulose	PROTEIN	PROTEIN	PROTEIN	[Cr]	DM	ASH
	Mcal/kg								total	bacterial	plant	ug/g		
FDPTN	FDEN	DDM	DASH	DOM	DNDF	DADF	DADL	DCLL	DPTNT	DPTNB	DPTNP	DCR	FDM	FASH
13.4767	4.236	0.0482	0.1729	0.8271	0.429	0.274	0.076	0.1988	21.9140	10.0076	11.9064	666.2	0.3485	0.1284
13.4767	4.236	0.0552	0.1566	0.8434	0.434	0.273	0.073	0.1996	21.0805	6.8804	14.2001	657.6	0.3917	0.1198
13.4767	4.236	0.0542	0.1417	0.8583	0.455	0.292	0.074	0.2172	21.1385	4.7200	16.4185	903.4	0.3869	0.1138
13.4767	4.236	0.0454	0.1719	0.8281	0.411	0.266	0.071	0.1950	22.4190	6.4860	15.9330	684.3	0.3809	0.1067
13.2443	4.220	0.0501	0.1642	0.8358	0.398	0.244	0.064	0.1795	23.6385	14.0231	9.6154	558.3	0.3753	0.1189
13.2443	4.220	0.0529	0.1616	0.8384	0.431	0.263	0.073	0.1904	22.0520	8.2810	13.7710	622.8	0.3587	0.1227
13.2443	4.220	0.0563	0.1521	0.8479	0.462	0.296	0.085	0.2109	19.5225	5.1333	14.3892	1025.1	0.3598	0.1358
13.2443	4.220	0.0424	0.1846	0.8154	0.417	0.262	0.072	0.1902	21.4275	8.7069	12.7206	578.2	0.4048	0.1264
14.0403	4.252	0.0408	0.2089	0.7911	0.329	0.194	0.058	0.1356	25.8055	22.2221	3.5834	416.0	0.3680	0.1262
14.0403	4.252	0.0501	0.1707	0.8293	0.394	0.239	0.066	0.1729	24.9415	10.7749	14.1666	541.9	0.3432	0.1194
14.0403	4.252	0.0392	0.2153	0.7847	0.321	0.201	0.055	0.1463	23.1180	5.0405	18.0775	849.8	0.4640	0.1139
14.0403	4.252	0.0351	0.2266	0.7734	0.326	0.200	0.063	0.1376	22.9140	7.6699	15.2441	599.4	0.3914	0.1417
14.1907	4.214	0.0366	0.2287	0.7713	0.302	0.177	0.055	0.1216	27.3185	15.9016	11.4169	455.3	0.4151	0.1220
14.1907	4.214	0.0468	0.1837	0.8163	0.394	0.238	0.071	0.1674	23.6725	10.6611	13.0114	604.5	0.3574	0.1434
14.1907	4.214	0.0364	0.2069	0.7931	0.314	0.190	0.046	0.1442	23.5190	14.3326	9.1864	583.5	0.3462	0.1112
14.1907	4.214	0.0324	0.2349	0.7651	0.329	0.203	0.059	0.1439	23.9440	10.6339	13.3101	407.4	0.4206	0.1122
13.738	4.231	0.047	0.184	0.816	0.403	0.252	0.074	0.179	22.006	8.368	13.638	723.786	0.364	0.137
13.738	4.231	0.043	0.196	0.804	0.369	0.228	0.064	0.164	23.236	9.132	14.104	635.208	0.419	0.121
13.738	4.231	0.045	0.187	0.813	0.386	0.238	0.066	0.172	23.235	11.464	11.771	587.423	0.389	0.119
13.738	4.231	0.046	0.178	0.822	0.379	0.235	0.062	0.173	23.630	11.404	12.225	592.005	0.361	0.114
13.738	4.231	0.044	0.194	0.806	0.365	0.222	0.063	0.159	24.669	15.539	9.131	523.955	0.382	0.124
13.738	4.231	0.051	0.168	0.832	0.413	0.253	0.071	0.183	22.937	9.149	13.787	606.706	0.363	0.126
13.738	4.231	0.047	0.179	0.821	0.388	0.245	0.065	0.180	21.825	7.307	14.518	840.449	0.389	0.119
13.738	4.231	0.039	0.205	0.795	0.371	0.233	0.066	0.167	22.676	8.374	14.302	567.312	0.399	0.122
13.477	4.236	0.051	0.161	0.839	0.433	0.276	0.073	0.203	21.638	7.023	14.615	727.870	0.377	0.117
13.244	4.220	0.050	0.166	0.834	0.427	0.266	0.073	0.193	21.660	9.036	12.624	696.101	0.375	0.126
14.040	4.252	0.041	0.205	0.795	0.343	0.208	0.060	0.148	24.195	11.427	12.768	601.796	0.397	0.125
14.191	4.214	0.036	0.214	0.786	0.335	0.202	0.058	0.144	24.614	12.882	11.731	512.655	0.385	0.122

211	212	213	214	215	216	217	218	219	220	221	222	223	224	225
							FEED COMPOSITION - dry matter basis							
OM	NDF	ADF	ADL	cellulose	PROTEIN Total	[Cr]	ASH	OM	NDF	ADF	ADL	cellulose	PROTEIN	ENERGY Mcal/kg
FOM	FNDF	FADF	FADL	FCLL	FPTNT	FCR								
0.8716	0.579	0.365	0.099	0.2660	13.1595	828.4	0.0932	0.9068	0.5770	0.3506	0.0535	0.2971	14.1268	4.4403
0.8802	0.584	0.368	0.105	0.2634	12.9395	1065.0	0.0932	0.9068	0.5770	0.3506	0.0535	0.2971	14.1268	4.4403
0.8862	0.591	0.369	0.098	0.2706	12.5355	1029.4	0.0932	0.9068	0.5770	0.3506	0.0535	0.2971	14.1268	4.4403
0.8933	0.572	0.354	0.101	0.2530	13.4435	932.5	0.0932	0.9068	0.5770	0.3506	0.0535	0.2971	14.1268	4.4403
0.8811	0.567	0.360	0.099	0.2606	13.6795	856.3	0.0922	0.9078	0.5752	0.3470	0.0528	0.2943	13.8678	4.4191
0.8773	0.569	0.364	0.111	0.2531	12.7275	973.3	0.0922	0.9078	0.5752	0.3470	0.0528	0.2943	13.8678	4.4191
0.8642	0.574	0.373	0.122	0.2503	12.2390	1157.9	0.0922	0.9078	0.5752	0.3470	0.0528	0.2943	13.8678	4.4191
0.8736	0.569	0.381	0.117	0.2640	12.4870	862.4	0.0922	0.9078	0.5752	0.3470	0.0528	0.2943	13.8678	4.4191
0.8738	0.577	0.358	0.110	0.2479	12.4985	875.6	0.0997	0.9003	0.5628	0.3376	0.0532	0.2844	14.7084	4.4545
0.8906	0.578	0.360	0.111	0.2484	13.1050	860.5	0.0997	0.9003	0.5628	0.3376	0.0532	0.2844	14.7084	4.4545
0.8831	0.585	0.366	0.104	0.2620	12.5680	1163.7	0.0997	0.9003	0.5628	0.3376	0.0532	0.2844	14.7084	4.4545
0.8583	0.568	0.361	0.113	0.2477	12.3285	950.0	0.0997	0.9003	0.5628	0.3376	0.0532	0.2844	14.7084	4.4545
0.8780	0.590	0.381	0.112	0.2691	12.1460	833.6	0.0986	0.9014	0.5415	0.3219	0.0501	0.2719	14.8858	4.4207
0.8566	0.560	0.364	0.123	0.2402	12.6700	946.2	0.0986	0.9014	0.5415	0.3219	0.0501	0.2719	14.8858	4.4207
0.8888	0.580	0.377	0.115	0.2624	12.8275	976.3	0.0986	0.9014	0.5415	0.3219	0.0501	0.2719	14.8858	4.4207
0.8878	0.586	0.374	0.115	0.2587	11.8605	894.8	0.0986	0.9014	0.5415	0.3219	0.0501	0.2719	14.8858	4.4207
0.863	0.570	0.366	0.115	0.251	12.599	970.61	0.096	0.904	0.564	0.339	0.052	0.287	14.397	4.434
0.879	0.587	0.374	0.110	0.265	12.560	981.19	0.096	0.904	0.564	0.339	0.052	0.287	14.397	4.434
0.881	0.581	0.366	0.109	0.258	12.356	943.29	0.096	0.904	0.564	0.339	0.052	0.287	14.397	4.434
0.886	0.574	0.363	0.106	0.256	13.264	906.39	0.096	0.904	0.564	0.339	0.052	0.287	14.397	4.434
0.876	0.578	0.366	0.105	0.261	12.871	848.48	0.096	0.904	0.564	0.339	0.052	0.287	14.397	4.434
0.874	0.573	0.364	0.112	0.251	12.861	961.25	0.096	0.904	0.564	0.339	0.052	0.287	14.397	4.434
0.881	0.582	0.371	0.110	0.261	12.568	1081.83	0.096	0.904	0.564	0.339	0.052	0.287	14.397	4.434
0.878	0.579	0.367	0.112	0.256	12.480	909.91	0.096	0.904	0.564	0.339	0.052	0.287	14.397	4.434
0.883	0.582	0.364	0.101	0.263	13.020	963.84	0.093	0.907	0.577	0.351	0.053	0.297	14.127	4.440
0.874	0.575	0.369	0.112	0.257	12.783	962.46	0.092	0.908	0.575	0.347	0.053	0.294	13.868	4.419
0.875	0.577	0.361	0.110	0.252	12.650	962.46	0.100	0.900	0.563	0.338	0.053	0.284	14.708	4.455
0.878	0.579	0.374	0.116	0.258	12.326	912.72	0.099	0.901	0.542	0.322	0.050	0.272	14.886	4.421

226	227	228	229	230	231	232	233	234	235	236	237	238	239	240
DUODENAL COMPOSITION - dry matter basis									FECAL COMPOSITION - dry matter basis					
ASH	OM	NDF	ADF	ADL	cellulose	PROTEIN	[Cr] DCR	DMflow g/d DFLOW	ASH	OM	NDF	ADF	ADL	cellulose
0.1774	0.8226	0.4403	0.2814	0.0775	0.2039	22.4817	683.5	408.82	0.1331	0.8669	0.6001	0.3790	0.1031	0.2759
0.1612	0.8388	0.4469	0.2805	0.0751	0.2055	21.6963	676.8	412.85	0.1245	0.8755	0.6070	0.3823	0.1087	0.2736
0.1451	0.8549	0.4665	0.2986	0.0761	0.2224	21.6502	925.2	302.00	0.1178	0.8822	0.6126	0.3823	0.1019	0.2803
0.1764	0.8236	0.4219	0.2729	0.0728	0.2001	23.0012	702.1	398.00	0.1105	0.8895	0.5924	0.3662	0.1042	0.2619
0.1674	0.8326	0.4058	0.2483	0.0654	0.1829	24.0909	569.0	491.08	0.1237	0.8763	0.5899	0.3740	0.1030	0.2710
0.1658	0.8342	0.4426	0.2700	0.0746	0.1953	22.6286	639.1	437.19	0.1292	0.8708	0.5995	0.3829	0.1164	0.2665
0.1557	0.8443	0.4732	0.3028	0.0869	0.2159	19.9866	1049.5	266.25	0.1411	0.8589	0.5963	0.3872	0.1271	0.2601
0.1885	0.8115	0.4258	0.2676	0.0734	0.1943	21.8842	590.5	473.21	0.1319	0.8681	0.6143	0.3979	0.1225	0.2754
0.2124	0.7876	0.3347	0.1968	0.0590	0.1378	26.2290	422.9	660.76	0.1308	0.8692	0.5974	0.3710	0.1142	0.2568
0.1731	0.8269	0.3994	0.2418	0.0665	0.1752	25.2827	549.3	508.64	0.1233	0.8767	0.5967	0.3716	0.1150	0.2566
0.2197	0.7803	0.3275	0.2051	0.0558	0.1493	23.5854	867.0	322.28	0.1185	0.8815	0.6083	0.3810	0.1085	0.2725
0.2307	0.7693	0.3324	0.2039	0.0638	0.1401	23.3304	610.3	457.86	0.1472	0.8528	0.5903	0.3751	0.1178	0.2573
0.2373	0.7627	0.3135	0.1835	0.0573	0.1262	28.3529	472.5	591.38	0.1265	0.8735	0.6118	0.3951	0.1162	0.2790
0.1910	0.8090	0.4096	0.2480	0.0738	0.1741	24.6214	628.7	444.45	0.1491	0.8509	0.5819	0.3781	0.1283	0.2498
0.2140	0.7860	0.3250	0.1966	0.0475	0.1491	24.3239	603.5	463.03	0.1157	0.8843	0.6027	0.3920	0.1191	0.2729
0.2442	0.7558	0.3423	0.2114	0.0618	0.1496	24.8864	423.5	659.84	0.1167	0.8833	0.6098	0.3887	0.1196	0.2691
0.189	0.811	0.414	0.259	0.076	0.184	22.61	743.0	394.3	0.143	0.857	0.592	0.380	0.119	0.261
0.202	0.798	0.378	0.234	0.065	0.169	23.88	651.7	449.9	0.125	0.875	0.610	0.380	0.114	0.275
0.192	0.808	0.397	0.244	0.068	0.176	23.85	602.7	514.9	0.124	0.876	0.605	0.381	0.113	0.268
0.183	0.817	0.388	0.240	0.063	0.177	24.17	606.0	465.2	0.118	0.882	0.595	0.376	0.110	0.266
0.199	0.801	0.374	0.228	0.065	0.163	25.29	537.0	538.0	0.129	0.871	0.600	0.380	0.109	0.271
0.173	0.827	0.425	0.260	0.073	0.188	23.56	623.5	450.8	0.131	0.869	0.596	0.379	0.117	0.262
0.184	0.816	0.398	0.251	0.067	0.184	22.39	861.3	338.4	0.123	0.877	0.605	0.386	0.114	0.271
0.210	0.790	0.381	0.239	0.068	0.171	23.28	581.6	497.2	0.127	0.873	0.602	0.382	0.116	0.266
0.165	0.835	0.444	0.283	0.075	0.208	22.21	746.9	380.4	0.121	0.879	0.603	0.377	0.104	0.273
0.169	0.831	0.437	0.272	0.075	0.197	22.15	712.0	416.9	0.131	0.869	0.600	0.386	0.117	0.268
0.209	0.791	0.348	0.212	0.061	0.151	24.61	612.4	487.4	0.130	0.870	0.598	0.375	0.114	0.261
0.222	0.778	0.348	0.210	0.060	0.150	25.55	532.0	539.7	0.127	0.873	0.602	0.388	0.121	0.268

241	242	243	244	245	246	247	248	249	250	251	252	253	254	255
			DAPA VALUES									Digestive	flow parameters	
PROTEIN	[Cr]	OMflow	DAPA analysis - as measured - Intial values include both DL and DC							duodenal	Feed intake	weigh	feed	feed DM
		g/d	(mg/g SAMPLE)			Protein	Bacterial	duodenal	fecal	bacteria	(kg)	back	intake	0.84616
		FFLOW	bacteria	duodenal	fecal	bacteria	Prtn/DAPA	mg/g DM	mg/g DM	mgBACP/g				
13.6448	859.0	325.30	4.115	0.869	0.798	47.403	115.21	0.8911	0.8277	102.668	1.40	0.00	1.40	0.8468
13.4393	1106.2	252.60	4.900	0.663	0.718	50.890	103.85	0.6819	0.7459	70.813	1.40	0.00	1.40	0.8468
12.9840	1066.3	262.06	5.558	0.549	0.504	47.795	85.99	0.5622	0.5220	48.343	1.40	0.00	1.40	0.8468
13.9195	965.5	289.40	2.432	0.564	0.559	27.985	115.08	0.5782	0.5792	66.544	1.40	0.00	1.40	0.8468
14.2239	890.3	313.84	1.307	0.835	0.726	21.942	167.87	0.8514	0.7554	142.915	1.40	0.00	1.40	0.8533
13.4031	1025.0	272.61	4.895	0.832	0.768	48.693	99.48	0.8542	0.8086	84.975	1.40	0.00	1.40	0.8533
12.7183	1203.2	232.23	4.357	0.472	0.580	47.346	108.67	0.4836	0.6023	52.553	1.40	0.00	1.40	0.8533
13.0282	899.8	310.55	2.631	0.513	0.761	44.674	169.81	0.5237	0.7940	88.925	1.40	0.00	1.40	0.8533
12.9494	907.2	307.99	1.987	1.034	0.561	42.700	214.85	1.0513	0.5816	225.868	1.40	0.00	1.40	0.8495
13.5360	886.8	314.39	3.292	0.910	0.575	38.997	118.45	0.9221	0.5941	109.223	1.40	1.50	1.29	0.8495
13.1730	1210.1	230.90	4.152	0.456	0.467	45.887	110.53	0.4653	0.4856	51.424	1.40	3.10	1.18	0.8495
12.8054	986.7	283.16	3.111	0.566	0.551	42.153	135.52	0.5763	0.5727	78.080	1.40	0.00	1.40	0.8495
12.5936	864.4	323.27	2.634	0.966	0.496	43.364	164.63	1.0025	0.5135	165.037	1.40	0.00	1.40	0.8350
13.1729	983.7	284.04	3.492	0.873	0.670	42.637	122.12	0.9080	0.6962	110.885	1.40	0.00	1.40	0.8350
13.3398	1015.3	275.21	1.705	0.653	0.491	37.386	219.32	0.6759	0.4997	148.231	1.40	3.00	1.19	0.8350
12.1323	931.0	300.14	2.702	0.736	0.670	38.909	144.12	0.7669	0.6975	110.524	1.40	0.00	1.40	0.8350
														0.84616
13.09	1008.2	281.2	3.768	0.695	0.650	44.986	120.39	0.715	0.675	86.05	1.400	0.000	1.400	0.846
13.06	1020.1	279.3	3.579	0.649	0.610	46.204	137.20	0.668	0.635	94.05	1.400	0.775	1.345	0.846
12.87	982.4	285.7	3.785	0.786	0.626	44.532	136.11	0.809	0.652	117.43	1.400	0.000	1.400	0.846
13.75	940.0	298.2	2.184	0.741	0.585	31.578	155.18	0.757	0.607	116.73	1.400	1.125	1.320	0.846
13.35	880.2	317.6	2.511	0.926	0.646	39.852	165.64	0.949	0.670	159.12	1.400	0.000	1.400	0.846
13.39	1000.9	280.9	4.145	0.819	0.683	45.304	110.97	0.842	0.711	93.97	1.400	0.375	1.373	0.846
13.05	1123.7	250.1	3.943	0.533	0.508	44.603	131.13	0.547	0.527	75.14	1.400	1.525	1.291	0.846
12.97	945.7	295.8	2.719	0.596	0.636	38.438	141.13	0.611	0.661	86.02	1.400	0.000	1.400	0.846
13.50	999.2	282.3	4.251	0.681	0.645	43.518	105.04	0.678	0.669	72.09	1.400	0.000	1.400	0.847
13.34	1004.6	282.3	3.297	0.663	0.709	40.664	136.46	0.678	0.740	92.34	1.400	0.000	1.400	0.853
13.12	998.2	284.1	3.135	0.741	0.539	42.434	144.84	0.754	0.559	116.15	1.400	1.150	1.318	0.849
12.81	948.6	295.7	2.633	0.808	0.579	40.582	162.55	0.838	0.602	133.67	1.400	0.750	1.346	0.835

256	257	258	259	260	261	262	263	264	265	266	267	268	269	270
	FEED INTAKE COMPOSITION AS g/day									DUODENAL FLOWS				
DM intak	DM intake													
corrOM	avgDM	ASH	OM	NDF	ADF	ADL	cellulose	PROTEIN	ENERGY	DM	ASH	OM	NDF	ADF
INDM		INASH	INOM	INNDF	INADF	INADL	INCELL	INPTN	INEN	DDM	DASH	DOM	DNDF	DADF
1185.6	1184.63	110.46	1075.13	684.05	415.67	63.405	352.261	167.5	5.26	408.82	72.54	336.29	179.99	115.06
1185.6	1184.63	110.46	1075.13	684.05	415.67	63.405	352.261	167.5	5.26	412.85	66.56	346.29	184.49	115.82
1185.6	1184.63	110.46	1075.13	684.05	415.67	63.405	352.261	167.5	5.26	302.00	43.82	258.18	140.87	90.17
1185.6	1184.63	110.46	1075.13	684.05	415.67	63.405	352.261	167.5	5.26	396.00	70.21	327.79	167.93	108.62
1194.7	1184.63	110.17	1084.49	687.12	414.59	63.044	351.550	165.7	5.28	491.08	82.19	408.88	199.28	121.96
1194.7	1184.63	110.17	1084.49	687.12	414.59	63.044	351.550	165.7	5.28	437.19	72.49	364.70	193.48	118.02
1194.7	1184.63	110.17	1084.49	687.12	414.59	63.044	351.550	165.7	5.28	266.25	41.45	224.79	125.99	80.62
1194.7	1184.63	110.17	1084.49	687.12	414.59	63.044	351.550	165.7	5.28	473.21	89.20	384.02	201.50	126.65
1189.3	1184.63	118.62	1070.65	669.32	401.46	63.258	338.207	174.9	5.30	660.76	140.31	520.45	221.14	130.06
1098.3	1093.97	109.55	988.71	618.09	370.74	58.417	312.324	161.5	4.89	508.64	88.03	420.61	203.16	122.97
1001.2	997.26	99.86	901.31	563.45	337.97	53.253	284.715	147.3	4.46	322.28	70.80	251.48	105.54	66.09
1189.3	1184.63	118.62	1070.65	669.32	401.46	63.258	338.207	174.9	5.30	457.86	105.65	352.22	152.21	93.36
1169.0	1184.63	115.32	1053.68	633.03	376.35	58.541	317.805	174.0	5.17	591.38	140.35	451.03	185.37	108.54
1169.0	1184.63	115.32	1053.68	633.03	376.36	58.541	317.805	174.0	5.17	444.45	84.91	359.55	182.03	110.21
990.1	1003.31	97.67	892.40	536.14	318.74	49.580	269.161	147.4	4.38	463.03	99.07	363.97	150.46	91.04
1169.0	1184.63	115.32	1053.68	633.03	376.35	58.541	317.805	174.0	5.17	659.84	161.13	498.71	225.89	139.50
1184.6	1184.6	113.6	1071.0	668.4	402.0	62.1	340.0	170.5	5.25	394.3	76.1	318.2	160.1	99.8
1137.6	1137.8	109.0	1028.7	641.9	386.1	59.6	326.6	163.6	5.04	449.9	91.7	358.2	169.2	104.3
1184.6	1184.6	113.6	1071.0	668.4	402.0	62.1	340.0	170.5	5.25	514.9	104.4	410.5	195.3	119.4
1117.1	1116.5	107.0	1010.2	631.4	379.9	58.6	321.3	160.5	4.95	465.2	84.9	380.3	180.2	111.1
1184.6	1184.6	113.6	1071.0	668.4	402.0	62.1	340.0	170.5	5.25	536.0	108.8	429.2	196.4	118.9
1161.9	1162.0	111.4	1050.5	655.6	394.3	60.9	333.5	167.2	5.15	450.8	78.0	372.8	190.8	116.8
1092.9	1092.5	104.5	988.3	617.7	371.7	57.3	314.4	156.9	4.85	338.4	63.8	274.6	130.7	82.0
1184.6	1184.6	113.6	1071.0	668.4	402.0	62.1	340.0	170.5	5.25	497.2	108.5	390.7	186.9	117.0
1185.6	1184.6	110.5	1075.1	684.0	415.7	63.4	352.3	167.5	5.26	380.4	63.3	317.1	168.3	107.4
1194.7	1184.6	110.2	1084.5	687.1	414.6	63.0	351.6	165.7	5.28	416.9	71.3	345.6	180.1	111.8
1119.5	1115.1	111.7	1007.8	630.0	377.9	58.5	318.4	164.7	4.99	467.4	101.2	366.2	170.5	103.1
1124.3	1139.3	110.9	1013.4	608.8	361.9	56.3	305.6	167.4	4.97	539.7	121.4	418.3	186.9	112.3

271	272	273	274	275	276	277	278	279	280	281	282	283	284	285
						FECAL FLOWS							OBSERVED FECA	
													WET g/d	DM/day
ADL	cellulose	PROTEIN	PROTEIN	PROTEIN	DM	ASH	OM	NDF	ADF	ADL	cellulose	PROTEIN		
DADL	DCELL	DPTNT	DPTNB	DPTNP	FDM	FASH	FOM	FNDF	FADF	FADL	FCELL	FPTN	g/d	FDM
31.68	83.37	91.91	41.97	49.94	325.30	43.31	281.98	195.20	123.27	33.54	89.74	44.39	1402	488.64
31.00	84.82	89.57	29.24	60.34	252.60	31.44	221.16	153.33	96.57	27.46	69.11	33.95	902	381.13
22.99	67.18	65.39	14.60	50.78	262.06	30.88	231.18	160.54	100.18	26.72	73.46	34.03	790	305.69
28.98	79.63	91.54	26.48	65.06	299.40	31.97	267.43	171.43	105.97	30.16	75.81	40.29	1038	395.42
32.14	89.82	118.30	70.18	48.12	313.84	38.81	275.03	185.15	117.38	32.32	85.06	44.64	1226	463.89
32.62	85.40	98.93	37.15	61.78	272.61	35.22	237.39	163.42	104.38	31.73	72.88	36.54	999	358.34
23.13	57.49	53.21	13.98	39.22	232.23	32.76	199.47	138.46	89.93	29.52	60.41	29.54	436	156.87
34.72	91.92	103.56	42.08	61.49	310.55	40.96	269.59	190.77	123.58	38.05	85.53	40.46	986	389.17
38.97	91.09	173.31	149.25	24.07	307.99	40.28	267.71	184.00	114.25	36.16	79.09	39.88	1255	486.97
33.84	89.13	128.60	55.56	73.04	314.39	38.76	275.63	187.60	116.82	36.15	80.66	42.56	1472	505.22
17.97	48.11	76.01	16.57	59.44	230.90	27.36	203.55	140.46	87.98	25.06	62.92	30.42	288	133.65
29.22	64.14	106.82	35.76	71.07	283.18	41.68	241.50	167.17	106.23	33.36	72.87	36.26	944	359.46
33.91	74.63	167.67	97.60	70.07	323.27	40.91	282.36	197.77	127.73	37.55	90.18	40.71	1060	440.03
32.82	77.40	109.43	49.28	60.15	284.04	42.34	241.70	165.28	107.39	36.45	70.65	37.42	896	320.27
21.98	69.05	112.63	68.64	43.99	275.21	31.84	243.37	165.87	107.88	32.77	75.11	36.71	774	267.96
40.80	96.71	164.21	72.93	91.28	300.14	35.04	265.10	183.03	116.67	35.89	80.78	39.41	977	410.92
29.21	70.60	90.34	35.25	55.09	291.2	40.02	241.2	166.5	106.7	33.22	73.49	36.90	919.5	333.8
29.40	74.87	109.20	46.37	62.83	279.3	35.17	244.2	170.6	109.0	32.03	76.94	36.38	814.0	333.5
33.84	85.59	125.46	68.48	56.98	285.7	35.35	250.3	172.7	108.9	32.37	76.50	36.71	1005.3	390.5
29.24	81.91	112.77	55.21	57.55	298.2	35.34	262.9	177.5	112.0	32.85	79.16	41.05	1130.0	408.1
34.18	84.73	137.80	89.75	48.05	317.6	40.83	276.8	190.5	120.7	34.84	86.02	42.41	1238.3	469.9
32.57	84.19	106.63	42.81	63.83	280.9	36.94	244.0	167.4	106.3	32.95	73.35	37.61	1072.3	386.2
21.52	60.46	76.81	28.45	48.36	250.1	30.71	219.4	151.3	96.5	29.52	67.97	32.67	572.0	216.0
33.43	83.60	116.53	44.31	72.22	295.8	37.41	258.4	178.1	113.1	34.37	78.75	36.35	986.3	393.7
28.67	78.75	84.60	29.07	55.53	292.3	34.40	247.9	170.1	108.5	29.47	77.03	39.16	1038.0	387.7
30.65	81.16	93.50	40.85	52.65	282.3	36.94	245.4	169.5	109.8	32.80	75.91	37.79	914.3	344.6
30.00	73.12	121.19	64.29	56.90	284.1	37.02	247.1	169.8	108.3	32.44	73.89	37.20	989.8	373.8
32.38	79.96	138.49	72.11	66.37	295.7	37.53	258.1	178.0	114.9	35.67	79.25	37.81	926.8	359.8

286	287	288	289	290	291	292	293	294	295	296	297	298	299	300
										Percent total digestibility as observed				
DM intak	digestibility													
		ASH	OM	NDF	ADF	ADL	cellulose	PROTEIN	DM	ASH	OM	NDF	ADF	ADL
INTAKE	DIG	FASH	FOM	FNDF	FADF	FADL	FCELL	FPTN	PTOTDM	PTOTASH	PTOTOM	PTOTND	PTOTAD	PTOTADL
1185.6	68.79	65.06	423.58	293.21	185.17	50.38	134.80	86.67	58.785	41.088	60.602	57.136	55.452	20.546
1185.6	69.54	44.95	316.18	219.20	138.06	39.26	98.80	48.53	69.540	59.304	70.592	67.955	66.785	38.081
1185.6	74.22	36.02	269.67	187.26	116.85	31.16	85.69	39.69	74.217	67.394	74.918	72.624	71.868	50.851
1185.6	66.65	43.69	351.73	234.24	144.79	41.21	103.58	55.04	66.648	60.450	67.284	65.757	65.167	35.004
1194.7	61.17	57.36	406.53	273.67	173.50	47.77	125.72	65.98	61.170	47.934	62.514	60.171	58.153	24.224
1194.7	70.00	46.29	312.05	214.81	137.21	41.70	95.51	46.03	70.005	57.982	71.226	68.737	66.904	33.850
1194.7	86.87	22.13	134.74	93.55	60.75	19.94	40.81	19.95	86.869	79.912	87.576	86.386	85.348	68.374
1194.7	66.59	52.65	346.52	245.20	158.65	48.91	109.94	52.00	66.587	52.208	68.048	64.314	61.686	22.422
1189.3	59.05	63.69	423.26	290.93	190.64	55.59	125.05	63.06	59.053	46.310	60.465	56.534	55.004	12.114
1098.3	54.00	62.28	442.93	301.47	187.72	58.10	129.62	69.39	53.999	43.144	55.201	51.226	49.365	0.546
1001.2	86.65	15.83	117.81	81.29	50.92	14.51	36.42	17.01	86.651	84.145	86.929	85.572	84.932	72.760
1189.3	68.93	54.37	315.09	218.10	136.60	43.53	95.07	47.31	68.934	54.192	70.571	67.414	65.478	31.188
1169.0	62.36	55.69	384.35	269.20	173.87	51.11	122.76	55.42	62.358	51.712	63.523	57.475	53.801	12.692
1169.0	72.60	47.74	272.53	186.36	121.09	41.09	80.00	42.19	72.603	58.602	74.136	70.562	67.825	29.803
990.1	72.94	31.00	236.96	161.50	105.04	31.91	73.13	35.75	72.935	68.262	73.447	69.877	67.046	35.640
1169.0	64.85	47.07	362.96	260.59	159.74	49.14	110.60	49.85	64.849	58.404	65.564	60.414	57.556	16.053
1184.6	71.80	47.33	286.49	197.80	126.40	38.73	87.67	44.03	71.798	58.444	73.221	70.374	68.526	37.478
1137.6	71.28	42.28	291.21	203.72	130.43	39.45	91.98	43.39	71.284	61.842	72.273	68.829	66.801	36.468
1184.6	67.03	46.49	341.89	235.90	148.61	44.40	104.21	50.16	67.031	57.522	68.041	64.577	62.838	28.217
1117.1	63.69	46.56	359.54	242.72	152.76	44.75	108.01	56.29	63.688	54.647	64.612	61.758	59.933	23.853
1184.6	60.34	60.45	409.43	281.75	179.30	51.21	127.08	65.79	60.342	46.764	61.776	57.829	55.602	17.394
1161.9	66.54	50.32	335.92	230.46	146.02	45.04	100.98	51.78	66.536	54.756	67.788	64.520	62.720	25.570
1092.9	80.17	26.24	189.79	130.90	83.39	24.38	59.01	29.25	80.169	74.928	80.717	78.615	77.304	56.908
1184.6	66.75	49.67	344.07	237.03	150.49	45.70	104.79	51.05	66.754	56.306	67.864	64.475	62.471	26.167
1185.6	67.30	47.43	340.29	233.48	146.22	40.50	105.72	52.48	67.297	57.062	68.349	65.868	64.823	36.120
1194.7	71.16	44.61	299.96	206.81	132.58	39.58	93.00	46.49	71.158	59.509	72.341	69.902	68.023	37.217
1119.5	67.16	49.05	324.79	222.95	139.47	42.93	96.54	49.09	67.159	56.940	68.291	65.186	63.695	29.152
1124.3	68.19	45.80	314.20	216.91	139.93	43.31	96.62	45.80	68.186	59.245	68.165	64.582	61.557	23.547

301	302	303	304	305	306	307	308	309	310	311	312	313	314	315
L FLOWS		DM Digestibilities based on ADL				flow	flow		Predicted Ruminal digestion % Using Duodenal composition					
cellulose PTOTCEI	PROTEIN PTOTPTN	Duodena	Fecal	rate	volume (L)	constant	coefficien	PRUMDM	ASH PRUMAS	OM PRUMOM	NDF PRUMNE	ADF PRUMAC	ADL PRUMAC	cellulose PRUMCE
61.734	60.191	31.00	48.13	-0.0471	16.00	3.424	-0.038	65.517	34.332	68.721	73.688	72.319	50.021	76.332
71.952	71.022	28.78	50.81	-0.0411	18.41	3.007	-0.022	65.177	39.744	67.790	73.030	72.136	51.104	75.921
75.674	76.303	29.75	47.54	-0.0538	11.68	3.022	-0.020	74.528	60.332	75.986	79.406	78.308	63.742	80.930
70.596	67.137	26.57	48.69	-0.0404	18.04	3.029	-0.024	66.430	36.441	69.511	75.450	73.869	54.283	77.394
64.237	60.172	19.37	48.76	-0.0375	18.96	3.199	-0.029	58.894	25.395	62.297	70.998	70.584	49.022	74.451
72.832	71.010	29.27	54.66	-0.0426	16.28	3.536	-0.042	63.405	34.202	66.372	71.842	71.533	48.259	75.706
88.392	87.957	39.26	58.48	-0.0565	11.28	3.639	-0.046	77.713	62.372	79.272	81.664	80.554	63.307	83.647
68.727	68.610	28.09	56.93	-0.0380	18.60	3.565	-0.041	60.389	19.036	64.590	70.675	69.452	44.920	73.852
63.026	63.950	9.81	53.41	-0.0489	16.38	3.375	-0.041	44.440	-18.285	51.389	66.960	67.605	38.396	73.068
58.497	57.665	20.06	53.75	-0.0497	15.52	3.215	-0.035	53.686	19.642	57.458	67.132	66.831	42.068	71.462
87.209	88.045	4.62	51.00	-0.0569	12.12	3.486	-0.048	67.810	29.099	72.099	81.269	80.446	66.250	83.101
71.891	72.953	16.64	54.85	-0.0427	16.93	3.524	-0.040	61.500	10.937	67.103	77.259	76.746	53.816	81.034
61.373	68.154	12.68	56.89	-0.0420	9.60	3.330	-0.025	49.411	-21.703	57.195	70.717	71.160	42.067	76.519
74.829	75.756	32.17	60.97	-0.0365	11.57	3.122	-0.021	61.980	26.373	65.877	71.245	70.715	43.944	75.646
72.831	75.746	-5.49	57.95	-0.0498	7.27	3.308	-0.027	53.232	-1.432	59.215	71.936	71.439	55.667	74.344
65.200	71.351	19.00	58.13	-0.0408	10.87	3.087	-0.017	43.555	-39.724	52.669	64.316	62.932	30.311	68.941
74.212	74.215	29.770	55.608	-0.046	13.945	3.427	-0.036	66.68	33.50	70.24	75.96	75.08	52.77	79.16
72.315	73.958	18.542	53.905	-0.044	14.684	3.347	-0.034	60.70	16.54	65.42	73.92	73.30	51.09	77.35
69.183	70.653	21.958	53.433	-0.047	13.803	3.255	-0.030	56.48	9.13	61.60	70.63	70.09	45.18	74.66
66.540	65.180	15.125	52.284	-0.044	14.951	3.188	-0.029	58.06	20.01	62.12	71.38	70.68	50.26	74.41
62.593	63.117	18.214	51.795	-0.044	15.237	3.332	-0.033	54.57	4.93	59.90	70.59	70.42	44.88	75.09
69.527	68.863	27.571	55.045	-0.042	15.445	3.220	-0.030	61.06	29.99	64.37	70.81	70.30	46.34	74.68
81.027	82.013	17.035	53.741	-0.054	10.590	3.364	-0.035	68.32	37.59	71.64	78.57	77.69	62.24	80.51
69.104	70.013	22.574	54.649	-0.040	16.111	3.301	-0.031	57.97	6.67	63.47	71.93	70.75	45.83	75.31
69.989	68.663	29.026	48.790	-0.046	16.033	3.120	-0.026	67.91	42.71	70.50	75.39	74.16	54.79	77.64
73.547	71.937	28.996	54.706	-0.044	16.281	3.485	-0.040	65.10	35.25	68.13	73.79	73.03	51.38	76.91
70.156	70.653	12.782	53.251	-0.050	15.239	3.400	-0.041	56.86	10.35	62.01	73.15	72.91	50.13	77.17
68.558	72.752	14.591	58.483	-0.042	9.829	3.212	-0.022	52.04	-9.12	58.74	69.55	69.06	43.00	73.86

316	317	318	319	320	321	322	323	324	325	326	327	328	329	330
			Predicted Total digestion % Using Estimated fecal values							Bacterial analysis Duodenal-ESTIMATED			efficiencies	
PROTEIN total L	PROTEIN plant PRUMPT	DM PTOTDM	ASH PTOTASI	OM PTOTOM	NDF PTOTND	ADF PTOTAD	ADL PTOTADI	cellulose PTOTCE	PROTEIN PTOTPTI	DM g/d ESDDM	App DM ESDAPP	True DM ESDTRU	Bac/App ESDBA	Bac/True ESDBT
45.123	70.184	72.56	60.788	73.772	71.464	70.343	47.106	74.526	73.499	88.55	776.76	688.21	113.99	128.66
46.519	63.974	78.69	71.534	79.429	77.585	76.767	56.688	80.381	79.731	57.45	772.73	715.28	74.34	80.32
60.962	60.679	77.90	72.047	78.497	76.531	75.900	57.865	79.146	79.685	30.55	883.59	853.04	34.57	35.81
45.342	61.155	75.59	71.054	76.056	74.938	74.506	52.431	78.480	75.948	94.64	787.59	692.95	120.16	136.57
28.592	70.953	73.73	64.775	74.640	73.055	71.689	48.735	75.805	73.055	319.85	703.59	383.73	454.60	833.53
40.286	62.710	77.18	68.035	78.110	76.217	74.822	49.677	79.332	77.946	76.29	757.47	681.18	100.72	112.00
67.880	76.326	80.56	70.263	81.607	79.846	78.310	53.181	82.816	82.172	29.55	928.41	898.86	31.83	32.88
37.492	62.892	74.01	62.819	75.142	72.237	70.192	39.645	75.670	75.579	94.19	721.45	627.25	130.56	150.17
0.921	86.242	74.10	66.043	74.996	72.509	71.541	44.415	76.615	77.200	349.52	528.51	178.99	661.34	1952.78
20.390	54.782	71.37	64.620	72.122	69.649	68.491	38.112	74.173	73.656	142.46	589.62	447.15	241.62	318.59
48.382	59.637	76.94	72.606	77.416	75.072	73.967	52.936	77.901	79.344	36.12	678.89	642.78	53.20	56.19
38.932	59.373	76.19	64.866	77.443	75.024	73.539	47.258	78.455	79.269	84.82	731.41	646.58	115.97	131.19
3.644	59.731	72.35	64.525	73.202	68.759	66.060	35.859	71.623	76.604	225.07	577.62	352.55	389.65	638.41
37.114	65.435	75.70	63.285	77.061	73.891	71.464	37.743	77.676	78.498	115.59	724.54	608.96	159.53	189.81
23.580	70.151	72.20	67.403	72.728	69.062	66.155	33.899	72.096	75.090	183.59	527.04	343.45	348.34	534.54
5.633	47.543	74.32	69.618	74.840	71.086	68.998	38.684	74.582	79.074	187.29	509.15	321.86	367.85	581.89
47.26	67.83	76.25	64.80	77.47	75.06	73.41	46.32	78.37	78.36	79.63	790.28	710.65	105.33	120.64
34.01	61.56	75.50	67.87	76.30	73.41	71.75	46.28	76.39	77.81	103.21	687.67	584.47	161.94	231.27
26.95	66.54	75.88	68.94	76.61	74.09	72.82	47.66	77.42	78.48	160.91	669.68	508.77	291.12	670.62
29.48	64.26	73.22	66.96	73.89	71.68	70.21	43.29	75.14	74.44	185.13	651.96	466.82	291.18	455.81
19.57	71.78	73.19	64.03	74.15	71.45	69.91	44.03	74.64	75.09	245.75	646.62	400.87	404.90	888.34
36.08	61.73	75.74	66.87	76.68	74.34	72.89	45.56	77.89	77.46	97.95	711.09	613.14	144.05	175.18
50.20	68.95	76.90	70.58	77.56	75.13	73.58	49.47	77.99	79.07	69.95	754.48	684.53	116.99	164.85
31.85	57.74	75.03	67.09	75.87	73.32	71.81	44.50	76.80	77.47	115.24	687.40	572.16	183.64	249.96
49.49	66.25	76.19	68.86	76.94	75.13	74.38	53.52	78.13	77.22	67.79	805.17	737.37	85.77	95.34
43.56	68.22	76.37	66.47	77.37	75.34	73.75	47.81	78.41	77.19	129.97	777.73	647.76	179.43	282.15
27.16	65.01	74.65	67.03	75.49	73.06	71.88	45.68	76.79	77.37	153.23	632.11	478.88	268.03	614.69
17.49	60.72	73.64	66.21	74.46	70.70	68.17	36.55	73.99	77.32	177.88	584.59	406.70	316.34	486.16

331	332	333	334	335	336	337	338	339	340	341	342	343	344	345
Fecal-ESTIMATED					Fecal-OBSERVED						g	Calorimetry		
DM g/d	App DM	True DM	Bac/App	Bac/True	DM g/d	App DM	True DM	Bac/App	Bac/True	Duodenal	analysis	O2	CO2	CH4
ESFDM	EDFAPP	ESFTRU	ESFBA	ESFBT	OBFDM	OBFAPP	OBFTRU	OBFBA	OBFBT	inlPPM	poolsize	mL/min	mL/min	mL/min
65.44	860.29	794.85	76.06	82.32	98.29	860.29	761.99	114.26	129.00	1723	270.3	531.85	579.86	31.10
38.45	932.98	894.53	41.21	42.98	54.97	932.98	878.01	58.92	62.60	1015	458.8	507.02	565.16	32.24
24.61	923.53	898.92	26.65	27.38	28.71	923.53	894.82	31.09	32.09	1051	443.1	339.55	561.02	28.67
68.93	896.19	827.26	76.91	83.32	94.18	896.19	802.01	105.09	117.43	1070	435.2	357.81	586.37	33.80
181.37	880.82	699.45	205.91	259.31	268.09	880.82	612.73	304.37	437.54	1581	294.6	582.54	447.61	19.02
45.04	922.05	877.02	48.84	51.35	59.20	922.05	862.85	64.20	68.61	3439	135.4	588.24	590.44	25.79
32.11	962.43	930.33	33.36	34.51	21.69	962.43	940.75	22.53	23.05	4356	106.9	471.24	436.23	17.04
93.72	884.11	790.39	106.00	118.57	120.46	884.11	763.65	136.25	157.75	3669	126.9	642.89	624.11	37.30
90.14	881.28	791.14	102.28	113.93	142.52	881.28	738.76	161.72	192.92	2373	196.2	609.71	632.49	33.71
56.73	783.87	727.14	72.37	78.02	91.17	783.87	692.71	116.30	131.61	1640	284.0	585.16	618.60	34.20
27.01	770.27	743.26	35.06	36.34	15.63	770.27	754.64	20.29	20.72	3065	151.9	423.58	458.97	19.18
52.14	906.09	853.95	57.54	61.06	68.02	906.09	838.07	75.07	81.17	3339	139.5	542.89	473.50	26.80
63.03	845.73	782.70	74.52	80.52	85.79	845.73	759.94	101.44	112.89	2137	217.9	729.19	469.77	33.21
56.64	884.95	828.32	64.00	68.37	63.86	884.95	821.10	72.16	77.77	1325	351.5	704.32	618.35	39.83
80.68	714.86	634.18	112.86	127.22	78.55	714.86	636.31	109.89	123.45	2034	229.0	632.66	517.80	25.80
77.48	868.86	791.37	89.18	97.91	106.08	868.86	762.77	122.09	139.07	1221	381.4	709.21	624.04	33.03
51.58	903.44	851.86	57.74	61.57	62.97	903.44	840.48	71.01	77.75	2686	217.03	562.58	526.99	28.69
55.55	858.27	802.72	64.20	69.60	69.21	858.27	789.06	79.23	88.49	2472	238.90	575.67	529.50	30.48
59.32	898.93	839.61	66.74	72.64	84.13	898.93	814.80	94.78	108.17	2021	289.04	561.66	602.00	30.30
96.93	818.94	722.01	117.01	136.97	133.00	818.94	685.94	158.91	202.51	1581	310.68	539.54	542.59	28.21
99.99	867.03	767.04	114.70	134.02	148.67	867.03	718.36	170.45	218.08	1954	244.75	613.32	532.43	29.26
49.21	880.97	831.75	56.61	60.18	67.30	880.97	813.67	77.90	85.15	1855	307.42	599.19	598.14	33.02
41.10	842.77	801.67	51.98	56.36	36.15	842.77	806.63	45.95	49.83	2627	232.73	466.76	493.51	22.67
73.07	888.81	815.74	82.41	90.21	97.19	888.81	791.63	109.63	123.85	2325	270.76	563.20	577.00	32.73
49.36	903.25	853.89	55.21	59.00	69.04	903.25	834.21	77.34	85.28	1215	401.86	434.06	573.10	31.45
88.06	912.36	824.30	98.53	115.94	117.36	912.36	795.00	131.84	171.74	3261	165.95	571.23	524.60	24.79
56.50	835.38	778.87	66.81	72.34	79.34	835.38	756.04	93.35	106.60	2604	192.91	540.33	545.89	28.47
69.46	828.60	759.14	85.14	93.51	83.57	828.60	745.03	101.40	113.30	1679	294.94	693.85	557.49	32.97

346	347	348	349	350
Energy losses		Protozoal populations		
CH4	total			
GELOSS	EPROD			
Mcal	Mcal	Day 0	Day 2	Day 14
0.294	2.736	210000	140000	210000
0.305	2.622	217000	231000	133000
0.271	1.971	266000	35000	35000
0.319	2.069	189000	7000	28000
0.180	2.779	182000	7000	14000
0.244	2.969	168000	21000	21000
0.161	2.336	168000	217000	175000
0.352	3.215	186667	63000	112000
0.319	3.099	49000	14000	28000
0.323	2.987	119000	14000	14000
0.181	2.178	182000	42000	63000
0.253	2.653	175000	154000	140000
0.314	3.366	161000	105000	70000
0.376	3.444	119000	140000	133000
0.244	3.054	133000	21000	21000
0.312	3.474	140000	49000	42000
0.27	2.79	168000	162750	164500
0.29	2.85	186667	110250	94500
0.29	2.88	155750	29750	31500
0.27	2.72	155750	12250	19250
0.28	2.99	150500	66500	80500
0.31	3.01	155750	101500	75250
0.21	2.38	187250	78750	73500
0.31	2.85	172667	68250	80500
0.30	2.35	220500	103250	101500
0.23	2.83	176167	77000	80500
0.27	2.73	131250	56000	61250
0.31	3.33	138250	78750	66500